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SOMMAIRE - INHALT - SOMMARIO - CONTENTS

M. FONTAINE: Quelques problèmes physiologiques posés par le *Salmo Salar*. Intérêt de l'étude de la *smoltification* type de préparation au comportement migratoire 433 Congressus 438

Brèves communications - Kurze Mitteilungen - Brevi comunicazioni - Brief Reports

- A. LONGINELLI: Age of the Pleochroic-Halos of the Quartz-Monzonite of Eastern Elba 439
- J. N. CHATTERJEA and H. MUKHERJEE: Cyclisation of α -Benzylhomophthalic Acids 439
- S. S. JANDE: Chromosome Mechanism in *Polididus armatissimus* (Reduviidae-Heteroptera). 440
- J. B. MORRILL, JR.: Concerning the Free Amino Acids in the Hydroid Tubularia 441
- M. ANBAR, I. AVIAD, R. REIN, and S. SCHORR: The Accumulation of 3-Iodo N, N, Diacet-6 Aminobenzoic Acid (IDAB) in Growth Areas of Bone 443
- G. LAUDAHN: Stabilisierung der oxydativen Phosphorylierung isolierter Mitochondrien durch artspezifische und artunspecifische Serumproteine 444
- U. S. SRIVASTAVA: Secretory Cycle and Disappearance of the Prothoracic Glands in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) 445
- A. ZINNARI: Activation of Pancreatic Trypsinogen by Liver and Kidney Mitochondria 446
- G. RINDI and L. DE GIUSEPPE: Thiamine and its Mono-, Di-, and Triphosphoric Esters Content of Normal Rat Tissues 447
- C. W. F. T. PISTORIUS: Synthesis at High Pressure and Lattice Constants of Normal Cupric Carbonate 447
- R. G. TSANEV and G. G. MARKOV: Nucleic Acid Content in Mouse Epidermis Separated from Dermis by Different Methods 448
- S. SCHWIMMER, J. F. CARSON, R. U. MAKOWER, M. MAZELIS, and F. F. WONG: Demonstration of Alinase in a Protein Preparation from Onion 449
- S. R. GUHA and H. S. CHAKRAVARTI: The Correlation between Anion-Induced Mitochondrial Swelling and Glutaminase I Activity of Guinea Pig Liver 451
- D. KRITCHEVSKY, J. LANGAN, and M. W. WHITEHOUSE: Distributions of Cholesterol Amongst Liver Subcellular Fractions 452
- V. PETERA and V. LAHN: Transaminasenaktivität nach Methyltestosteron-, Testosteronpropionat-, Chlorpromazin- und Methylthiouracilbehandlung 453
- K. BLAIM: Einfluss der Wurzeln auf die Trigonellinsynthese bei *Coffea arabica* 454
- F. HEĚMANSKÝ and J. VÍTEK: The Role of Proconvertin and Stuart Factor in the Inactivation of Tissue Thromboplastin by Serum 455
- H. KONZETT and R. A. BOISSONNAS: Biological Properties of Synthetic Bradykinin 456
- I. NOVOTNÝ and V. KUBIŠTA: The Effect of Uncoupling Agents on Metabolism of Insect Muscle 457
- B. C. R. STRÖMBLAD: Cholinesterase Activity in Skeletal Muscle after Botulinum Toxin 458
- BRIGITTA HOLMQUIST: Crossed Reflex Actions Evoked by High Threshold Muscle Afferents 459
- M. HAMBURGH: Observations on the Neuropathology of 'Reeler', a Neurological Mutation in Mice 460
- E. COSTA, S. GARATTINI, and L. VALZELLI: Interactions between Reserpine, Chlorpromazine, and Imipramine 461
- B. KONIECZNA-MARCZYNSKA and A. SKOWRON-CENDRAK: Hematological and Serological Investigations in Heteroparabiosis 463
- B. NIKODIJEVIĆ and S. VANOV: The Influence of Thenalidine on Reserpine- and Serotonin-Induced Gastric Ulcers in Rats 464
- V. SCHREIBER, M. RYBÁK, and V. KMENTOVA: Effect of Non-Protein Fraction of Rabbit Hypothalamic Extract on the Function of Adenohypophyseal Autografts in the Anterior Chamber of the Eye of Hypophysectomized Rats 466
- E. GIACOBINI, S. IZIKOWITZ, and A. WEGMANN: The Urinary Excretion of Noradrenaline and Adrenaline during Acute Alcohol Intoxication in Alcoholic Addicts 467
- H. U. D. WIESENDANGER and R. A. PASTERNAK: An Ultra-High Vacuum System Using an Oil-Diffusion Pump with a Non-Refrigerated Isolation Trap (Pro Experimentis) 467
- H. BRINTZINGER, B. PRIJS and H. ERLENMEYER: Zur Rolle von Schwermetallionen im Wirkungsmechanismus von Strahlenschädigung und Strahlenschutz. (Cogitationes) 468
- C. SCHLIEFER, H. FLÜGEL, and J. RUDOLF: Temperature and Salinity Relationships in Marine Bottom Invertebrates. (Cogitationes) 470

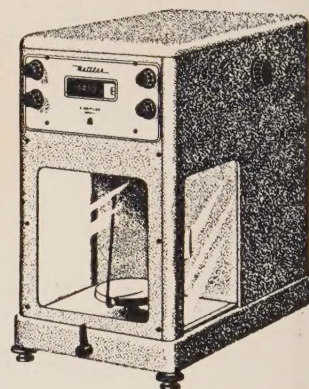


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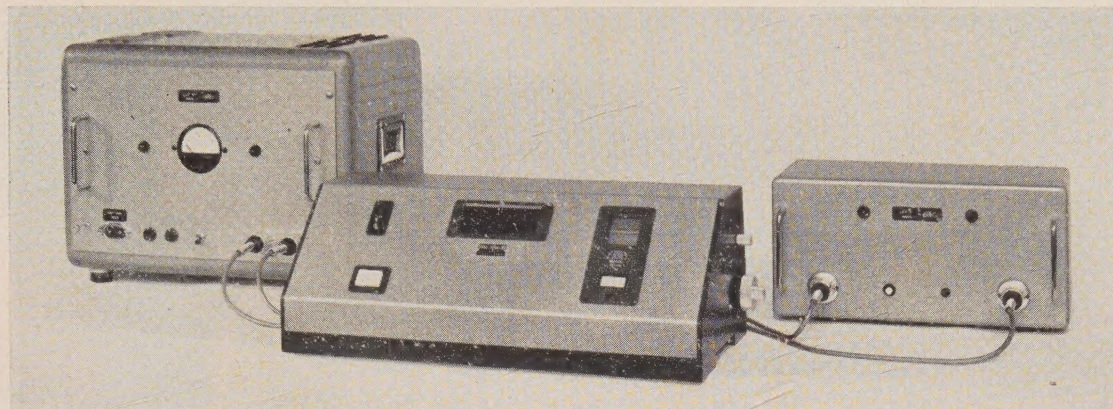
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in Vorbereitung

Quelques problèmes physiologiques posés par le *Salmo Salar*. Intérêt de l'étude de la *smoltification* type de préparation au comportement migratoire

Par M. FONTAINE *

Le comportement migratoire pose de nombreux problèmes. Celui du *Salmo Salar* est particulièrement stimulant, en lui-même et par les particularités du cycle vital qui lui sont liées et qui offrent au physiologiste des conditions favorables pour tenter de répondre à de nombreuses questions, d'une portée très générale.

Voici un jeune poisson, naissant dans nos rivières, effectuant la première partie de sa croissance sous un aspect fort voisin de celui de la truite commune (parr) et qui, plus ou moins rapidement, s'en distingue par une évolution de la robe, de la croissance, du comportement (smolt); il quitte alors nos eaux douces. Parvenu en mer, il migre vers une aire d'engraissement souvent lointaine, où sa croissance devient intense. Puis, à la suite d'une évolution physiologique – qui peut se situer dans le temps à un moment très différent selon les individus (automne, hiver, printemps) – voici que survient une crise physiologique d'une ampleur et d'une durée véritablement exceptionnelles. Le saumon cesse de s'alimenter, se rapproche des côtes, pénètre dans le fleuve de son enfance et gagne sa frayère natale ou un lieu très proche de celle-ci, et c'est là qu'il va se reproduire. Par quels mécanismes est suspendu l'appétit du saumon? Nous ne pouvons actuellement donner de réponse justifiée. Certains auteurs, recherchant les facteurs métaboliques de l'appétit des mammifères, avaient pensé faire intervenir la glycémie, d'autres la teneur en acides aminés du sang. Une hyperglycémie pour les uns (CARLSON, notamment), une amino-acidémie élevée pour les autres (MELLINKOFF), entraînerait une réduction de l'appétit. Mais ces hypothèses, qui ont d'ailleurs reçu diverses critiques, ne semblent pas pouvoir s'appliquer au jeûne physiologique du *Salmo Salar*. Les *smolts*, qui s'alimentent abondamment, ont une glycémie moyenne de 90 mg de glucose pour 100 cm³ de sang; les *parrs* mâles mûrs sur les frayères, qui consomment des quantités souvent considérables d'oeufs de saumon, ont une glycémie de 102, alors que les saumons adultes qui frayent et qui jeûnent, présentent des

glycémies de 80 environ. La seule donnée qui pourrait être considérée comme favorable à la présente hypothèse est la glycémie du *mended* qui, capturé peu de temps avant qu'il quitte les eaux douces et retourne en mer où il va se nourrir abondamment, présente une valeur de 42 seulement¹.

Quant à l'hypothèse de MELLINKOFF *et al.*, selon laquelle le taux d'acide aminé du plasma réglerait l'appétit, elle ne peut trouver confirmation chez le saumon. Les teneurs en acides aminés du plasma des *parrs* sur les frayères, *parrs* qui s'alimentent, sont du même ordre de grandeur que celles des saumons adultes qui jeûnent (1bis). La teneur la plus élevée est trouvée pour le *smolt* (13,88 mg %₀₀ N), qui manifeste cependant un vif appétit.

Il ne semble pas que la maturation génitale puisse être directement en cause dans l'apparition de ce jeûne physiologique, puisque les saumons de l'Adour pénètrent dans le fleuve avant que se manifeste un développement sensible des gonades. Par contre, on peut penser qu'il existe une certaine relation entre le métabolisme des lipides et la suspension de l'alimentation, puisque celle-ci coïncide avec une accumulation considérable de lipides dans l'organisme du saumon et que la reprise de l'alimentation, chez le *mended* parvenant en eau de mer, correspond à un état de profond amaigrissement. Mais les rapports, s'ils existent, restent fort imprécis. Il faut d'ailleurs souligner le point très important établi par MISLIN² dans son travail fondamental sur le saumon du Rhin, à savoir que le jeûne physiologique spontané du saumon, désigné par cet auteur sous le nom de jeûne synchronique, diffère du jeûne imposé par de multiples caractères qu'il a parfaitement mis en lumière. Voilà donc une étape du cycle vital qui pose un

* Laboratoires de Physiologie du Museum National d'Histoire Naturelle et de l'Institut Océanographique, Paris.

¹ M. FONTAINE et J. HATEY, *Physiol. comp. oecol.* 3, 37 (1953). – M. FONTAINE, Résultats inédits.

² H. MISLIN, *Revue suisse Zool.* 48, 1 (1941).

problème très intéressant, dont la solution – ou même l'approche – éclairerait certainement la question générale si débattue du mécanisme de l'appétit.

On peut, avec étonnement, se demander comment, en dépit d'un jeûne aussi prolongé, le saumon peut conserver jusqu'à la fraye sa surprenante activité. Sans doute, du fait de mécanismes neuro-endocriniens particulièrement bien réglés et puissants. Nous avons pu montrer la richesse du milieu intérieur du saumon à cette étape, en deux groupes d'hormones dynamogènes, les hormones thyroïdiennes et certaines hormones corticosurrénales, les 17 hydroxycorticostéroïdes. En ce qui concerne ces dernières, la teneur du plasma du saumon est, exprimée en 17 hydroxycorticostérone, de 50 mcg pour 100 cm³ environ au début de sa migration de montée et, sur les frayères, de 30³. Rappelons que, chez l'homme, cette même teneur est voisine de 15.

Du plasma du milieu intérieur du saumon capturé au cours de sa migration anadrome, la teneur moyenne en iode est de 124 mcg pour 100 cm³ de sérum, en iode lié aux protéines de 31, en iode thyroïdien de 16⁴. Sur les frayères, ces valeurs sont respectivement de 21.5, 15.7, 6.04. Ainsi la teneur du plasma en iode hormonal diminue beaucoup moins que l'iode total. Ce sont là des valeurs élevées, si nous les comparons à celles obtenues chez les mammifères ou chez certains poissons marins peu actifs (tels *Scorpoena* et *Pleuronectes*), et qui expliquent en partie le dynamisme surprenant du saumon. Mais comment n'y a-t-il pas épuisement des éléments chimiques constitutifs des hormones en jeu? La question paraît se poser avec une particulière acuité pour l'iode, puisque le saumon passe tout ce long jeûne synchronique dans une eau pauvre en cet élément. Il est probable que cette économie de l'iode – de l'iode accumulé au préalable au cours de la vie dans le milieu marin – est réalisée par divers mécanismes limitant des pertes et notamment par la particularité suivante: contrairement à ce qui est observé chez les mammifères et chez plusieurs espèces de poissons sédentaires – ou migrateurs thalassotoques, telle l'Anguille – les iodures sont liés dans le plasma du saumon à une certaine fraction protéique du milieu intérieur⁵. Sans doute cette liaison est-elle labile, puisqu'elle est détruite par l'acide trichloracétique, mais il est bien nécessaire qu'il en soit ainsi pour que l'iode puisse être utilisé par la glande thyroïde, et il est probable que les protéines plasmatiques en jeu, en liant les iodures résultant du catabolisme précédant l'anabolisme intense qui se fait au niveau des organes génitaux, limitent les pertes d'iodures au niveau de la branchie. Ainsi, peu à peu, quelques coins du voile qui couvre la physiologie du grand migrateur, se soulèvent.

A l'issue de ce long jeûne, survient donc la reproduction. Soulignons que, si la migration anadrome peut se dérouler à des époques très différentes de l'année, la fraye est au contraire limitée à quelques semaines. La maturation des gonades s'accélère chez les salmonidés

au cours d'une période de diminution d'illumination, alors que chez un grand nombre d'autres espèces de téléostéens cette maturation est corrélative d'un accroissement de l'illumination. A quelles différences physiologiques correspondent ces différences de sensibilité au facteur lumière? Nous l'ignorons, mais c'est là encore une question d'ordre général, car on observe des différences de même nature dans d'autres groupes zoologiques. Après la fraye, certains saumons succombent, les plus épuisés, semble-t-il; d'autres, après avoir gardé un moment encore la robe de fraye (*kelt*), la transforment en une robe argentée (*mended*) et gagnent une seconde fois les eaux marines où ils vont à nouveau s'engraisser, périr sous la dent des prédateurs ou revivre une seconde fois frayer en eau douce.

Comment se déclenchent ces divers mouvements migratoires? Probablement⁶ par une étape critique de ce conflit permanent entre l'organisme vivant et son milieu, de ce conflit qui représente la vie. Mais dans quelles conditions l'étudier? Il faut évidemment connaître les principaux caractères de la physiologie du poisson dans son état sédentaire, dans sa condition pré-migratrice et au cours de sa migration. Ainsi aurons-nous quelque chance de saisir les modifications qui ont sensibilisé l'organisme à certains facteurs externes, déclenchant ainsi le grand voyage. Quel type de migration choisir pour cette étude? La migration reproductrice? Dans les eaux marines françaises, rares et assez imprévisibles sont les captures des saumons sur le point d'entreprendre ce voyage. D'ailleurs, sans doute ne serait-il pas toujours facile de distinguer les individus encore sédentaires de ceux «prémigrateurs», prêts à partir au moindre stimulus ou de ceux qui ont réellement commencé leur migration vers les fleuves. Aussi, nous avons choisi d'étudier ce problème chez le jeune saumon sédentaire, puis préparant sa migration vers la mer, et enfin migrant. Cette étape présente le grand avantage d'être accompagnée, chez le saumon de l'Adour, de transformations de la morphologie externe si nettes que l'état prémigratoire est bien marqué. Nous n'avons jamais vu migrer, en effet, un seul jeune saumon qui n'ait subi ces modifications connues sous le nom de *smoltification*. C'est pourquoi nous avons fait porter une grande partie de nos efforts et de ceux de nos collaborateurs sur le mécanisme physiologique de cette évolution.

Rappelons-en d'abord les principaux traits. Nous trouvons, dans les eaux de nos fleuves, de jeunes saumons à robe marquée de taches sombres et de vives couleurs rappelant celles de la truite: ce sont les *parrs*. Au printemps, un certain nombre de ceux-ci subissent

³ M. FONTAINE et J. HATEY, C. R. Acad. Sci. 239, 319 (1954).

⁴ M. FONTAINE et J. LELOUP, C. R. Acad. Sci. 230, 775, 1216 (1950).

⁵ M. FONTAINE et J. LELOUP, C. R. Acad. Sci. 247, 767 (1958).

⁶ M. FONTAINE, Rapports du Colloque sur l'Instinct, organisé par la Fondation Singer-Polignac (Masson, 1956), p. 151.

une phase d'accélération de croissance, prennent une livrée argentée, accentuent la pigmentation mélanique de leurs nageoires, puis entreprennent la migration catadrome: ils sont dits *smolts*. Dans le gave d'Oloron – que nous avons spécialement étudié – la transformation du *parr* en *smolt* (*smoltification*) précède toujours cette migration. Elle apparaît comme une condition essentielle et non comme la conséquence de cette migration qui, selon l'opinion de LA ROCHE, LEBLOND et PREFONTAINE, conduisant les *parrs* dans des eaux riches en iode, leur permettrait d'élaborer les quantités d'hormone thyroïdienne nécessaires à cette *smoltification*⁷. En France, la *smoltification* s'effectue en eau douce.

Des observations analogues aux nôtres ont été faites par ROBERTSON⁸ à l'occasion des migrations holobiotiques de la truite arc-en-ciel. Certains individus de cette espèce, vers l'âge de deux ans, se réunissent dans les parties basses des fleuves où ils sont nés et subissent un profond changement de robe, les lignes et les taches sombres de la jeune truite sédentaire étant remplacées par cet éclat argenté, dû au dépôt de guanine sur les écailles, qui est le caractère le plus frappant de la *smoltification*. Dès que cette transformation, corrélative d'une hyperactivité thyroïdienne, est achevée, les individus qui l'ont subie migrent dans un grand lac. Cependant, il est des truites qui restent dans ces fleuves toute leur vie, qui ne migrent pas vers les lacs; ce sont celles qui ne prennent pas la livrée argentée. Chez cette espèce également, une *smoltification* semble bien conditionner le comportement migratoire.

Si l'on veut bien songer enfin que la transformation du *kelt* en *mended* rappelle beaucoup la *smoltification* du jeune saumon, puisqu'elle se manifeste par un changement de robe très similaire, il semble bien que – chez les salmonidés en eau douce – cette *smoltification* apparaisse comme l'expression particulièrement frappante de cette préparation physiologique au comportement migratoire que nous cherchons à comprendre.

Je rassemblerai ici quelques uns de nos résultats, relatifs à cette préparation physiologique, et tenterai de les interpréter.

HOAR, le premier, a montré⁹ que la thyroïde du *smolt* du *Salmo Salar* L. de l'Amérique du Nord présente, à l'étude histologique, des caractères d'hyperactivité par rapport à celle du *parr* (jeune saumon sédentaire). Nous avons étendu ces résultats au *Salmo Salar* du Bassin de l'Adour, précisé les modalités de cet hyperfonctionnement par l'étude physico-chimique de la fonction thyroïdienne (dosage chimique de ¹²⁷I, utilisation de ¹³¹I)¹⁰ et montré que si le comportement de lutte dans un courant (comportement familier du *smolt* en migration catadrome) entraîne bien, chez la truite arc-en-ciel, un hyperfonctionnement thyroïdien par rapport au comportement dans une eau calme¹¹, la *smoltification* naturelle d'un jeune saumon sédentaire s'accompagne déjà d'une importante accélération du fonctionnement thyroïdien, préalable donc à la migration catadrome¹².

Devant la difficulté d'obtenir, dans les gaves pyrénéens, de jeunes saumons *smoltifiés*, mais pas encore migrants, aux temps précédant immédiatement la migration, de jeunes *parrs* mâles, participant en décembre à la fraye des grands saumons adultes, étaient chaque année capturés sur les frayères et transportés dans l'étang voisin d'une Station expérimentale des Eaux et Forêts. (Grâce à la collaboration de M. l'Ingénieur R. VIBERT et de son personnel que nous remercions vivement). Au cours des deux ou trois mois qui suivaient, une partie des individus se *smoltifiait*, cependant qu'une autre partie conservait la robe du *parr*. Nous pouvions donc comparer les animaux parvenus à l'état prémigratoire, mais encore sédentaires, à ceux restés à l'état de *parrs*. Nous nommerons les premiers *smolts* sédentaires, réservant le nom de *smolts* aux individus capturés en cours de migration catadrome. Or, chez ces *smolts* sédentaires, on observe, tant par les critères histologiques que par les critères physico-chimiques (emploi du radioiode), les indices d'une hyperactivité très nette de la glande thyroïde. A cette hyper-sécrétion d'hormone thyroïdienne, il ne semble guère douteux qu'il faille rattacher l'argenture de la *smoltification*, de nombreux auteurs ayant montré l'influence des hormones thyroïdiennes sur le dépôt de guanine dans les écailles. Une étape biochimique de l'argenture des écailles a été mise en évidence par SVARD¹³, qui a montré les fluctuations de la teneur du sang en triphosphate de guanosine et en monophosphate d'inosine au cours de la *smoltification* du jeune saumon.

Il existe probablement aussi un lien entre cet hyperfonctionnement thyroïdien et, d'une part, la chute du glycogène hépatique¹⁴, d'autre part l'augmentation de la consommation d'O₂¹⁵ qui accompagnent la *smoltification*. En effet, si de nombreux auteurs n'ont pas observé d'augmentation du métabolisme respiratoire sous l'influence des hormones thyroïdiennes chez les téléostéens, certains autres ont constaté une telle action et il semble bien que, sous certaines conditions – peut-être action prolongée, synergie d'action avec un certain état neuroendocrinien – l'hormone thyroïdienne puisse être efficace.

Selon toute vraisemblance, cette hyperactivité thyroïdienne est d'ailleurs dépendante – en partie du moins – d'une stimulation hypophysaire, qui se manifeste sous la forme d'une dégranulation des cellules

⁷ G. LA ROCHE, C. P. LEBLOND et G. PREFONTAINE, Rev. can. Biol. 9, 101 (1950).

⁸ O. H. ROBERTSON, Physiol. zool. 21, 282 (1948).

⁹ W. S. HOAR, J. Morphol. 65, 257 (1939).

¹⁰ M. FONTAINE et M. OLIVEREAU, C. R. Acad. Sci. 224, 1660 (1947). – M. FONTAINE, J. LELOUP et M. OLIVEREAU, Arch. sci. physiol. 6, 83 (1952).

¹¹ M. FONTAINE et J. LELOUP, C. R. Acad. Sci. 249, 343 (1959).

¹² M. FONTAINE et J. LELOUP, Arch. sci. physiol. 14, 15 (1960).

¹³ P. O. SVARD, Nature 182, 1448 (1958).

¹⁴ M. FONTAINE et J. HATEY, J. Physiol. com. oecol. 3, 37 (1953).

¹⁵ M. FONTAINE et M. M. BARADUC, C. R. Soc. Biol. 149, 1327 (1955).

cyanophiles¹⁶⁻¹⁷. Et si, comme l'a très justement souligné EVROPEIZEVA¹⁸, il y a opposition entre smoltification et maturation génitale, c'est sans doute que l'hypophyse du jeune saumon utilise les glycoprotéides à sa disposition, soit pour l'élaboration de T. S. H., soit pour l'élaboration de gonadostimulines, mais ces deux hypersécrétions, qui exigent des composés de structure chimique très voisine, ne peuvent être assurées simultanément. On constate d'ailleurs également chez le saumon adulte cette régression de l'activité thyroïdienne, concomitante de la maturation génitale¹⁰.

Il faut rappeler ici certaines observations faites sur les saumons du Pacifique qui peuvent, à première vue, sembler en désaccord avec ce que nous avançons touchant le saumon atlantique de nos régions, mais qui pourront peut-être relever d'une explication commune. En effet, l'étude par HOAR et BELL¹⁹ de diverses espèces d'*Oncorhynchus* sur les côtes du Pacifique a conduit ces auteurs aux remarques suivantes: O. KISUTCH et O. NERKA montrent une nette *smoltification* et une activité thyroïdienne correspondant au moment de la migration d'avalaison. Par contre, O. KETA et O. GORBUSCHA ne manifestent pas, lors de la migration d'avalaison et à la seule étude histologique, d'activité thyroïdienne nette. Mais il faut noter que ces poissons migrent vers la mer très jeunes, dès qu'ils émergent du lit de la rivière, et qu'ils présentent déjà à ce moment une robe argentée²⁰. On peut donc supposer qu'ils ont eu auparavant, très précocement, une phase d'hyperactivité thyroïdienne préparant leur organisme au comportement migratoire. Les migrants tardifs présentent, d'ailleurs, une hyperplasie thyroïdienne qui pourrait représenter une réponse glandulaire à un épuisement des hormones thyroïdiennes secrétées dans la première phase d'activité. Cette hypothèse devrait être évidemment soumise à vérification par l'emploi conjugué des techniques biochimiques et histologiques.

Comment cet état d'hyperfonctionnement thyroïdien peut-il préparer le comportement migratoire? On sait que, chez les mammifères, les hormones thyroïdiennes (thyroxine et triiodothyronine) augmentent l'excitabilité du système nerveux central par un double mécanisme: stimulation directe (mise en évidence aussi chez les téléostéens par HOAR *et al.*²¹) et stimulation indirecte par augmentation de la sécrétion des hormones cortico et médullo-surréaliennes. Il est bien démontré, chez les mammifères, que ces hormones surréaliennes favorisent l'activité motrice générale de l'organisme, et les travaux récents de GONCHAROVA montrent que la cortisone provoque une élévation considérable de l'excitabilité du système nerveux²². Or, chez le smolt sédentaire, on note déjà une activation de l'interrénal et une hypertrophie de ce tissu glandulaire par les critères cytologiques et histologiques, mais ces phénomènes s'intensifient chez le smolt²³ et l'on observe, à ce stade, une corticostéroïdémie exceptionnellement élevée²⁴. Il est permis de supposer que l'hyperactivité interrénali-

enne est secondaire à une hypersécrétion hypophysaire d'hormone somatotrope et d'hormone corticotrope (cette hypersecrétion étant rendue très vraisemblable par l'augmentation du pourcentage des cellules fuchsinophiles lors de la smoltification et leur dégranulation^{16, 17} et, plus spécialement pour l'hormone somatotrope, par l'accélération de croissance, pour l'hormone corticotrope par le développement de la mélanogenèse), mais aussi à l'hyperfonctionnement thyroïdien. Ces divers facteurs hormonaux (corticotrope, somatotrope, thyrotrope, thyroïdien) agissent en effet, chez les mammifères, dans le sens d'un développement de la surrénale et de son activité fonctionnelle.

Nous pensons donc que cet ensemble de phénomènes neuroendocriniens aboutit à une hyperexcitabilité qui joue son rôle dans la préparation à la migration, car celle-ci semble bien se manifester d'abord par une agitation motrice qui conduit le smolt à quitter le lit du fleuve où il trouvait des abris contre un courant souvent violent. Ce smolt nage en plein courant; on le voit fréquemment sauter hors de l'eau, et il est vraisemblablement plus sensible aux stimuli de ce courant. De plus, l'imprégnation de l'organisme par les hormones thyroïdiennes fait sans doute du smolt un organisme très météorosensible qui répondra à des variations de certains facteurs auxquelles restent indifférents les parrs.

Mais les stimulines hypophysaires, les hormones thyroïdiennes, les hormones interrénaliennes ne sont pas seules en cause. Peu à peu se situent, dans des rôles différents, les divers facteurs de la *smoltification*. Il semble bien qu'il faille faire intervenir aussi l'hormone ou les hormones mélanophorétiques. On sait en effet que le smolt présente une mélanisation très nette, bien visible notamment dans les nageoires pectorales. Ce phénomène traduit sans doute une sécrétion accrue d'hormone mélanophorétique. Or, GUILLEMIN et KRIVOV²⁵ ont tout récemment montré, chez le mammifère, que l'hormone mélanophorétique augmente l'amplitude des potentiels évoqués au niveau d'un arc réflexe monosynoptique et exerce un effet de facilitation. Une hypersécrétion d'hormone mélanophorétique chez le parr-smolt n'est donc vraisemblablement pas étrangère à une sensibilité accrue de son système nerveux aux

¹⁶ M. FONTAINE et M. OLIVEREAU, C. R. Acad. Sci. 228, 772 (1949).

¹⁷ M. OLIVEREAU, Ann. Inst. Océan. 29, 195 (1954).

¹⁸ N. W. EVROPEIZEVA, Rapp. et P. V. Cons. perm. Internat. Explor. Mer 148, 29 (1959).

¹⁹ W. S. HOAR et G. M. BELL, Canad. J. Res. D. 28, 126 (1950).

²⁰ Communication de W. S. HOAR.

²¹ W. S. HOAR, O. MAC KINNON et A. REDLICH, Canad. J. Zool. 30, 273 (1952). — W. S. HOAR, M. M. A. KEENLEYSIDE et R. G. GOODALL, Canad. J. Zool. 33, 428 (1955).

²² V. N. GONCHAROVA, Bjull. eksper. Biol. Med. S.S.S.R. 48, n° 9, 84 (1959).

²³ M. FONTAINE et M. OLIVEREAU, J. Physiol. 49, 174 (1957); Bull. Soc. zool. France 84, 161 (1959).

²⁴ M. FONTAINE et J. HATEY, C. R. Acad. Sci. 239, 319 (1954).

²⁵ R. GUILLEMIN et W. A. KRIVOV, C. r. Acad. Sci. 250, 1117 (1960).

stimuli du milieu ambiant. Cette hypothèse nous semble d'autant plus intéressante à retenir que, chez d'autres migrateurs, nous observons aussi cette augmentation de la mélanisation, juste avant la manifestation d'un comportement migratoire actif, en particulier chez la jeune anguille ou civelle immédiatement avant et pendant sa migration anadrome. D'autre part, nous avons vu les raisons que nous possédions de supposer, à ce stade, une augmentation de la sécrétion d'hormone somatotrope hypophysaire. Or il apparaît que celle-ci joue un rôle important dans le contrôle du métabolisme hydrominéral des vertébrés et notamment des salmonidés. De ceux-ci, elle accroît beaucoup l'euryhalinité²⁶ et augmente la teneur en K de leur cerveau et de leurs muscles²⁷.

Précisément, chez le smolt sédentaire aussi bien que chez le smolt migrant, on note, dans le muscle et dans le cerveau, une augmentation de la teneur en K et une diminution du rapport K intracellulaire/K extracellulaire²⁸. Il semble bien que cette augmentation de la teneur en K soit due en grande partie à une hypersécrétion d'hormone somatotrope, toutefois, la diminution du rapport du K intracellulaire au K extracellulaire peut être attribuable à l'hyperfonctionnement thyroïdien, puisque TIMIRAS et WOBBURY²⁹ l'ont observée dans le cerveau du rat à la suite d'injections de thyroxine et de triiodothyronine.

Ces modifications de l'équilibre ionique entraînent sans doute des variations d'excitabilité qui jouent un rôle important dans la sensibilisation de l'organisme à certains facteurs externes: l'une des faces de cet état prémigratoire que nous tentons de préciser.

Il faut envisager aussi, dans l'ensemble de ces transformations, l'intervention du produit de neurosécrétion du tractus hypothalamo-hypophysaire et, d'une façon plus hypothétique, de celui observé dans l'organe sous-commissural. Pendant la *smoltification*, en effet, et aussi pendant la transformation de *kelt* en *mended*, les cellules du noyau préoptique se vident de leur produit de neurosécrétion et celui-ci, par cheminement axonal, gagne la neurohypophyse³⁰. On constate, d'autre part, lors de la *smoltification*, une augmentation du neurosécrétat dans l'organe sous-commissural³¹, et des arguments ont été développés en faveur de la nature différente du produit de neurosécrétion d'origine hypothalamique et du produit d'origine sous-commissurale. Le premier joue sans doute un rôle dans les modifications hydrominérales observées lors de la *smoltification* et dans l'augmentation de l'euryhalinité constatée à ce stade³². Il est difficile toutefois de préciser ce rôle, de récentes recherches tendant à montrer que les hormones hypothalamo-neurohypophysaires des téléostéens ne sont pas toutes identiques à celles des mammifères, comprenant notamment, sans doute, une arginine vasotocine qui n'existe pas chez ceux-ci, et l'expérimentation effectuée avec vasopressine ou ocytocine devant être reprise avec les hormones mêmes des pois-

sons. Le rôle du second est encore très mystérieux, car nous n'avons pas observé de disparition du produit lors du transfert des smolts en eau de mer.

On voit, par ces quelques exemples, combien la *smoltification* est un état particulier, très précieux pour l'étude de la préparation physiologique à la migration, puisqu'elle nous désigne les individus aptes à entreprendre la grande migration – avant tout changement de milieu, avant toute activité itinérante.

Une autre espèce de poisson migrateur amphibiotique – mais thalassotoque – l'anguille, présente, avant la migration d'avalaison, diverses modifications de la morphologie externe, dont l'argenture qui rappelle impérieusement celle présente dans la *smoltification* du jeune saumon. On retrouve alors l'hyperactivité de la thyroïde³³, et cette convergence de manifestations d'hyperfonctionnement endocrinien nous paraît assez convaincante de l'intervention de la thyroïde dans la préparation au comportement migratoire.

Par contre, il ne semble pas que les glandes génitales et leurs hormones jouent un rôle important dans ces phénomènes, car très variés sont les états des glandes génitales au moment de la migration. Les anguilles migrent alors que les organes génitaux viennent de commencer à se développer, mais que leur maturation semble stoppée – alors que les smolts femelles migrent avec des glandes génitales au repos. En ce qui concerne les smolts mâles, des états très variés peuvent être observés, les uns présentant des testicules filiformes et n'ayant subi aucune maturation génitale au cours de leur vie en eau douce, les autres ayant participé quelques mois plus tôt à la fraye, et, parmi ceux-ci, certains présentant des testicules encore latescents, d'autres des testicules ayant considérablement régressé. Cependant, cette opinion ne résulte que d'observations macroscopiques et il apparaît qu'une étude histologique de ces gonades de divers stades devrait être entreprise, à l'image de ce qui a été fait par EVROPEIZEVA¹⁸ sur le saumon de la Baltique.

Nombreux sont les poissons migrateurs qui nous sont accessibles pendant leur migration, mais à ce moment nous ne pouvons plus discerner de façon certaine, parmi les différences observées entre l'animal sédentaire et l'animal migrant, celles qui furent vraiment des facteurs primaires du comportement migratoire, car il en est qui manifestement résultent, du moins pour une grande part, non pas d'une préparation physiologique

²⁶ D. C. W. SMITH, Mem. Soc. Endocrinol. 5, 83 (1956).

²⁷ M. M. CHARTIER-BARADUC, C. R. Soc. Biol. 153, 1757 (1959).

²⁸ M. M. CHARTIER-BARADUC, Bull. Centre Et. Rech. sci. Biarritz, sous presse (1960).

²⁹ P. S. TIMIRAS et D. M. WOBBURY, Endocrinology 58, 181 (1956).

³⁰ L. ARVY, M. FONTAINE et M. GABE, C. R. Ass. Anat., 42^e Réunion 233, 225 (1955).

³¹ L. ARVY, M. FONTAINE et M. GABE, Arch. Anat. micr. Morphol. exp. 44, n° 4 (1955).

³² L. ARVY, M. FONTAINE et M. GABE, J. Physiol. 51, 1031 (1959).

³³ O. CALLAMAND et M. FONTAINE, Arch. Zool. exp. gén. 82, 129 (1942). – C. BERNARDI, Riv. Biol. Ital. 40, 186 (1948).

interne préalable à la migration, mais de la lutte dans le fleuve, à contre courant. Ainsi, par exemple, avons-nous constaté, chez la truite vivant dans un courant rapide, une diminution du Cl et du Na du muscle par rapport à la truite vivant en eau calme³⁴, diminution que nous avons aussi constatée du stade parr sédentaire au stade smolt migrant, mais que nous ne trouvons pas chez le smolt sédentaire^{28,35}. Voici pourquoi il est si important de pouvoir étudier un état prémigrateur bien défini, tel celui du smolt encore sédentaire.

Il est intéressant aussi de souligner que, par certains aspects au moins, la préparation physiologique à la migration et la migration elle-même apparaissent comme comportant des fluctuations de fonctionnement physiologique ou de valeurs biochimiques de sens opposé à celles qui résulteront du changement de milieu faisant suite à la migration catadrome. Par exemple, les signes histologiques d'une stimulation thyroïdienne constatée lors de la smoltification régressent lors du passage d'eau douce en eau de mer¹⁷. L'augmentation du rapport K/Na dans le cerveau et le muscle du smolt est évidemment corrigée dans un milieu comme l'eau de mer, exceptionnellement riche en Na et relativement pauvre en K.

Ainsi, la migration catadrome apparaît-elle, dans son aboutissement, comme un mécanisme biologique compensateur – du moins provisoirement – d'un déséquilibre physiologique survenu à un stade précis du développement. Ce déséquilibre physiologique de l'animal au moment même de sa migration se manifeste de diverses façons et notamment par le coefficient de variation élevé de certaines données biochimiques^{10,35,36}, par des variations de la natrémie particulièrement amples, lors du passage d'eau douce en eau de mer³⁷.

Les quelques faits rassemblés ci-dessus montrent que l'étude de l'évolution physiologique prémigratoire, quand celle-ci nous est accessible et bien marquée par des caractères manifestes, doit nous conduire vers une connaissance meilleure des mécanismes intimes des comportements migratoires et de leurs significations profondes. C'est pourquoi nous avons insisté ici sur la *smoltification*, manifestation très précieuse de cette évolution physiologique prémigratrice, qui représente l'une des étapes capitales d'une migration.

Summary

The author considers some physiological problems raised by particularities of the physiological cycle of *Salmo salar* L. about which he and his fellow-workers have produced new data (especially those brought out by the physiological, normal fasting so particular to the adult S: The synchronic fasting of Mislin).

He insists on the importance, for studies on the physiological mechanism of migrations and from the methodological point of view, of the following feature of the young Salmon in fresh water: a transformation which, in the population studied (S. s. of Adour waters), is so characteristic of and tightly bound to the preparation to catadromic

migration that it marks the subjects ready for migration and makes it possible to particularise the new physiological conditions accompanying the phenomenon of migratory instinct (activation of thyroid and interrenal function, of some pituitary neurosecretions and secretions, metabolic changes...). By studying simultaneously: smoltified but not yet migrant fishes, smolts during migration, and a salmonid fish subjected to a current in conditions simulating those of migrating smolts, it is now possible to begin to distinguish the physiological features bound to the preparation for migration, from those resulting from migratory activity.

³⁴ M. FONTAINE et M. M. CHARTIER-BARADUC, résultats inédits.

³⁵ M. FONTAINE, C. R. Acad. Sci. 232, 2477 (1951).

³⁶ M. FONTAINE, *L'Instinct* (Masson éd., 1956), p. 154.

³⁷ H. J. KOCH, *Nature* 4, 682 (1959).

CONGRESSUS

Sweden

International Congress on Biophysics

Stockholm, July 31 to August 4, 1961

An International Congress on Biophysics will be held in Stockholm from July 31 to August 4, 1961. The purpose of the meeting is to provide a forum for international communication in the field of biophysics. Participants may include members of national societies of biophysics, medical physics, and related fields, and other scientists interested in pure and applied biophysics. The meeting will be divided between a series of symposia devoted to special topics in biophysics and to presentations of a number of contributed papers in pure and applied biophysics submitted by the participants.

Further information can be obtained from Dr. Bo LINDSTRÖM at the Department of Medical Physics, Karolinska Institutet, Stockholm 60, Sweden.

NOTA

Announcement. Molecular biophysicists are becoming increasingly aware of the biological significance of fast transfer processes utilizing elementary particles (electrons and protons). To inquire into the possible physiological role of fast transfer reactions in eliciting specific interactions in ordered macromolecular structures, a series of seminar lectures was held at the Massachusetts Institute of Technology during the spring term of 1960. Included were lectures on electron and energy transfer mechanisms (KASHA, TAUBE, MURRELL, WEBER, STRYER) and on current concepts of proton transport mechanisms (EIGEN and GRUNWALD) of immediate biophysical and biochemical significance. Properties of water and ice crucial for proton transfer were reviewed (FRANK, BRADY, KLOTZ, FERNANDEZ-MORAN). Six lectures of a more general nature dealt with aspects of macromolecular interaction properties (GERGELY, BERENDSEN, ONCLEY, DAVISON, WIENER, ELSÄSSER).

Abstracts of these lectures and pertinent references have been compiled and are available *gratis*. Requests should be sent to Professor F. O. SCHMITT, Department of Biology, Massachusetts Institute of Technology, Cambridge 39 (Mass.).

Brèves communications - Kurze Mitteilungen Brevi comunicazioni - Brief Reports

Les auteurs sont seuls responsables des opinions exprimées dans ces communications. - Für die kurzen Mitteilungen ist ausschliesslich der Autor verantwortlich. - Per le brevi comunicazioni è responsabile solo l'autore. - The editors do not hold themselves responsible for the opinions expressed by their correspondents.

Age of the Pleochroic-Halos of the Quartz-Monzonite of Eastern Elba

A complete geological and petrographical study of the granodioritic intrusive rocks of the isle of Elba has recently been carried out by MARINELLI¹. The Elba intrusions are localized, the one in the western part of the island (Monte Capanne), the other, smaller and a little more acidic, in the south-eastern zone, near Porto Azzurro. While the age of the western intrusion is geologically rather well known², this is not the case with the eastern one.

The Monte Capanne granodiorite has already been studied by the pleochroic halos method by DEUTSCH *et al*³, while such research has never been carried out on the Porto Azzurro one. The determination of the age of this rock is particularly interesting both in relation to the neighbouring intrusion only a few kilometers away, and in relation to the other age-determinations carried out on the Tuscan-archipelago and continental granites⁴.

Despite the limitations of the pleochroic-halos method in age-determinations, these recent rocks are particularly well-suited to this kind of research. In fact we are interested in the relative chronology of the various granitic intrusions which may be related to the magmatic activity following the Appenninic orogenesis⁵.

Besides, the recent age of these magmatic rocks allows us to detect relatively small differences in age, as the pleochroic-halos method allows us to determine age ratios rather than absolute ages.

The results have been obtained by studying a sample from the cores of a drilling carried out by Soc. "Montecatini" near Porto Azzurro, at a depth of about 275 m. This sample has been kindly given to me by MARINELLI, and described by him in the previously mentioned work. Some of the accessory minerals included in the biotite, with specific activities varying from 0.11 to 0.24 $\alpha/\text{cm}^2\text{sec}$, show no appreciable halos. The experimental points of the halos observed around the minerals with higher activities are plotted in the diagram.

The values of the D parameter of the optical density of the halos expressed in μm are plotted on the ordinate; the values of the specific activities of the corresponding emitting minerals are plotted on the abscissa. The points related to the halos of the Monte Capanne granodiorite are plotted on the same diagram, together with the calculated isochrones.

The sensitivity of the eastern Elba biotite to artificial irradiation, compared to that of the Monte Capanne biotite, has been experimentally studied using a Po^{210} source. (The details of the technique used will be described in a further note.) The sensitivity of the two biotites has been found to be practically identical. An examination of the experimental points and their related isochrones, leads us to conclude that there is no appreciable age difference between the two rocks.

It follows that the small quartz-monzonitic stock partially outcropping near Porto Azzurro, must be regarded as chronologically related to the same magmatic phase from which the Monte Capanne pluton originated.

A. LONGINELLI

C. N. R. N., Laboratorio Geologia Nucleare, Università di Pisa (Italia), March 31, 1960.

Riassunto

Nel quadro di una serie di ricerche tendenti a determinare le età relative delle rocce granitiche dell'arcipelago toscano e della Toscana continentale, è stata studiata con il metodo degli aloni pleocroici la quarzomonzonite di Porto Azzurro (isola d'Elba).

In base ai risultati ottenuti, riportati nel grafico, si può asserire che non vi è apprezzabile differenza di età tra la roccia studiata e la vicina granodiorite del Monte Capanne.

¹ G. MARINELLI, Atti Soc. tosc. Sci. nat. 66, 50 (1959).

² L. TREVISAN, Boll. Soc. geol. ital. 70, 435 (1951).

³ S. DEUTSCH, D. HIRSCHBERG, and E. PICCIOTTO, Bull. Soc. belge Géol. 65, 267 (1956).

⁴ S. DEUTSCH and A. LONGINELLI, Exper. 8, 15 (1959).

⁵ G. MERLA, Boll. Soc. geol. ital. 70, 95 (1951).

Cyclisation of α -Benzylhomophthalic Acids

During the course of experiments on the synthesis of dibenzotropones, the cyclisation of some α -benzylhomophthalic acids were examined. α -Veratrylhomo-phthalic acid¹ (I), on treatment with polyphosphoric acid, readily afforded the keto acid (II, m. p. 211°C) which on decarboxylation to (III, m. p. 135°C) followed by dehydrogenation² with N-bromosuccinimide gave 2:3-dimethoxydibenzotropone (m. p. 131–132°C). The structure of (III) is confirmed by its independent synthesis from veratrylidene-phthalide³ following the synthetic course of TREIBS and KLINKHAMMER².

When 3:4-diethoxybenzylhomophthalic acid (IV, m. p. 169–170°C), readily available by reduction of 3:4-diethoxybenzylidenhomophthalic acid (m. p. 178–179°C), was cyclised a mixture of the keto acid (V, m. p. 165°C) and

¹ NG. PH. BUU HOI, C. R. Acad. Sci., Paris 218, 942 (1944).

² W. TREIBS and H. J. KLINKHAMMER, Chem. Ber. 84, 671 (1951).

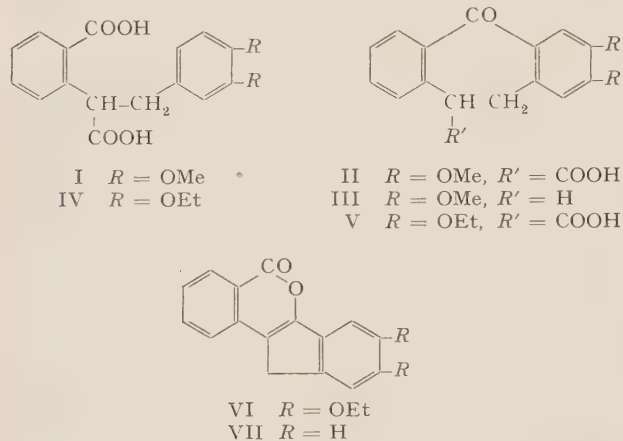
³ K. KODAMA, J. pharm. Soc. Japan 63, 54 (1943).

⁴ J. N. CHATTERJEA, Chem. Ber. 91, 2636 (1958).

⁵ W. BONTHORNE and D. H. REID, J. chem. Soc., London 1959, 2773.

an alkali-insoluble compound, $C_{20}H_{18}O_4$ (m. p. 176–177°C) resulted. The latter is identified as the diethoxyindenoisocoumarin (VI). α -Benzylhomophthalic acid itself afforded only the isocoumarin (VII, m. p. 173°C) on cyclisation. This structure is confirmed by the sharp lactone carbonyl absorption at 5.78μ (KBr pellet) and by conversion into 1:2-Benzo-4-azafluorene (m. p. 163–164°C; picrate, m. p. 234–235°C) by conventional methods. Further, reduction of the isocoumarin (VII) by lithium aluminium hydride⁴ gave the related isochromene (m. p. 103–104°C) which was readily converted into indeno-(3':2'-3:4)-isobenzopyrylium perchlorate (m. p. 197–199°C) by treatment with triphenylmethylperchlorate in acetic acid⁵.

Other substituted benzylhomophthalic acids so far examined have afforded only the indenoisocoumarins.



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February 17, 1960.

Résumé

Les cyclisations des acides α -benzylhomophthaliques ont produit un keto-acide à 7 termes, un indenoisocoumarin ou un mélange des deux.

Chromosome Mechanism in *Polididus armatissimus* (Reduviidae-Heteroptera)

The genus *Polididus* has so far been known^{1,2} cytologically by only a single species, *Polididus armatissimus*. The diploid number of chromosomes in this species is twelve, which includes a typical XY pair of sex chromosomes. The latter are of almost equal size and behave post-reductionally during meiosis. This chromosome number is the lowest among all the reduviids so far investigated which are otherwise characterised by the presence of a multiple X chromosome and a high number of chromosomes varying from twenty-three to thirty-two. Only in a few exceptional cases is the X simple. On account of the simple XY sex-determining mechanism and the lowest number of chromosomes, the *Polididus* constitutes an interesting material for cytological investigations.

During the studies on the chromosomes of Indian Heteroptera, the author³⁻⁵ happened to examine *Polididus armatissimus* which was found to differ markedly in its karyotype from that already described^{1,2}.

At the spermatogonial metaphase (Fig. 1) there are fourteen chromosomes out of which two pairs can be distinguished as rod-shaped from the remaining ten more or less round ones. The eight elements at the metaphase of the first meiotic division (Fig. 2) form the typical reduviid pattern—the six autosomal bivalents forming a ring, slightly outside which are the two sex chromosomes, X and Y. The sex chromosomes reveal only a slight difference in size among themselves. The metaphase of the second meiotic division (Fig. 3) also presents six autosomal elements forming a ring surrounding the centrally placed 'pseudo-bivalent' formed by X and Y. The two components of the 'pseudo-bivalent' differ slightly in their size (Fig. 4).

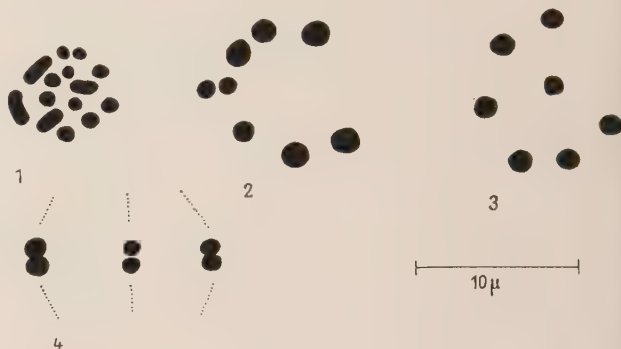


Fig. 1. Spermatogonial metaphase (polar view). Fig. 2. Metaphase I (polar view). Fig. 3. Metaphase II (polar view). Fig. 4. Metaphase II (side view), only three elements are shown.

Utilising the chromosome data to establish the phylogenetic relationship in the various families of Heteroptera, MANNA⁶ and BANERJEE² conclude that the number '12 + XY' represents the primitive karyotype in Heteroptera and that the evolution of Reduviidae from Lygaeoidea has been accompanied by an increase in the basic number of chromosomes and a change from a simple X to multiple X condition. Such an assumption would point to the primitive nature of the karyotype in the genus *Polididus*, where the '12 + XY' condition appears to have survived as such. From the facts that, in the various species of the family Reduviidae with multiple X chromosome, the size of the Y is quite large as compared with those of the Xs, and that the sizes of the individual Xs decrease as their number increases, it may be concluded, as already suggested by TROEDSSON⁷, that the increase in the number of Xs in the family has been accomplished by the fragmentation of the original X. The more or less equal size of X and Y in the genus *Polididus*, thus further supports the primitive nature of its karyotype amongst the reduviids.

S. S. JANDE⁸

Department of Zoology, Panjab University, Hoshiarpur (India), February 23, 1960.

¹ TOSHIOKA, Konchu 10 (1936). Quoted from S. MAKINO, *An Atlas of Chromosome Numbers in Animals* (Iowa State College Press, Ames 1950).

² M. R. BANERJEE, Proc. zool. Soc. 11, 9 (1958).

³ S. S. JANDE, Res. Bull. Panj. Univ. 10, 25 (1959).

⁴ S. S. JANDE, Res. Bull. Panj. Univ. 10, 215 (1959).

⁵ S. S. JANDE, Res. Bull. Panj. Univ., 10, 415 (1959).

⁶ G. K. MANNA, Proc. 10th Int. Congr. Ent. Canada 2, 919 (1956).

⁷ P. H. TROEDSSON, J. Morph. 75, 103 (1944).

⁸ Now at Chandigarh.

Résumé

Le nombre diploïde des chromosomes du *Polididus armatissimus* est de 14. L'X et l'Y sont de taille presque égale. L'ensemble des chromosomes de cette espèce représente un karyotype primitif pour la famille des Reduviidae.

Concerning the Free Amino Acids in the Hydroid Tubularia¹

Recently FAULHABER and TARDENT² demonstrated the presence of fourteen free amino acids in alcohol extracts of two to three hydranths of *Tubularia larynx*. Only six of these amino acids were detected in extracts of 40–60 regenerated hydranths and 3 mm long segments of hydrocauli. These results suggest that relatively few free amino acids are present in tubularian tissues and that marked qualitative differences in the distribution of free amino acids may exist between the morphologically distinct hydranth and hydrocaulus. The results also suggest that the free amino acid composition of hydranths recently regenerated from hydrocaulus tissues is more like that of the hydrocaulus than that of mature hydranths.

The present report is also concerned with the qualitative distribution of free amino acids and related substances in the mature hydranth, regenerated hydranth, and hydrocaulus of *Tubularia*. The results, contrary to those of FAULHABER and TARDENT, suggest that there are few if any qualitative differences in the free amino acid composition of the hydrocaulus and the hydranth.

Materials and Methods. Hydranth and hydrocaulus tissues of *Tubularia crocea* and *T. spectabilis* were prepared for alcohol extraction of free amino acids by first removing the hydranth and then the distal 4 mm of the hydrocaulus. The latter was discarded. The remaining unencrusted portions of the hydrocauli and the hydranths were frozen, lyophilized, and stored at -20°C . The nitrogenous substances were extracted from the lyophilized tissues with 80% ethanol according to AWAPARA³. Each extract was centrifuged, filtered, and mixed in a separatory funnel with three volumes of chloroform. After standing, the aqueous layer was drawn off and evaporated to dryness. The residue was taken up in 10% isopropanol and the nitrogen content adjusted to 11–12 mg N/ml. In order to detect as many substances as possible 4, 8, 12, 16, and 20 μl quantities of the extracts were chromatogrammed.

Chromatograms also were prepared of live tissues which were rinsed first in $10^{-3} M$ ethylenediamine tetraacetic acid (EDTA) in sea water, then in $10^{-3} M$ EDTA in distilled water, and applied directly to the paper and crushed⁴. The following amounts of material were chromatogrammed in this way: 5–10 mature hydranths with gonophores, 30–180 regenerated hydranths which lacked gonophores and actinula larvae, and 30–50 hydrocaulus segments 1 cm long which were first homogenized and then chromatogrammed.

Amino acids were separated by one- and two-dimension descending paper partition chromatography, using Whatman number 1 filter paper. The solvent system used for one-dimensional chromatograms was 1-butanol:acetic acid:water (4:1:1). With two-dimensional chromatograms this solvent was employed in the first direction and phenol:water:8-hydroxyquinoline (80:20:04) in the second direction. The amino acid spots were revealed by dipping the chromatograms in a solution of 0.5% ninhydrin in acetone. The spots were identified by comparing their

ninhydrin color and R_f 's with those of known amino acids on chromatograms prepared simultaneously.

Certain amino acids were identified further by treating one-dimensional chromatograms of hydranths and hydrocauli with other reagents. The sulfur amino acids—cysteine, cystine, methionine—were detected with sodium nitroprusside reagents⁵; arginine with the Sakaguchi reaction and nitroprusside reagent⁶; proline with isatin⁷; tyrosine and histidine with modified Pauly's reagent⁸; the hydroxyamino acids—serine and threonine—with the Nessler-periodate test⁶; and hydroxyproline by first treating the chromatogram with 0.2% isatin in acetone and then spraying with Ehrlich's reagent⁹. Tryptophane was detected by its fluorescence under ultraviolet light.

Taurine was not identified, nor were α - and β -alanine distinguished as was done in the study of FAULHABER and TARDENT².

Results. The following sixteen free amino acids were detected on chromatograms of crushed hydranths of *T. crocea*: alanine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, hydroxyproline, leucines, lysine, methionine, proline, serine, threonine, tyrosine, and valine. Chromatograms of *T. crocea* crushed hydrocauli were similar but lacked hydroxyproline, proline, cystine, and methionine.

Chromatograms of alcohol extracts of *T. crocea* revealed five additional free amino acids in hydranth tissues—arginine, asparagine, histidine, phenylalanine, and tryptophane. Chromatograms of alcohol extracts of hydrocauli and of crushed regenerated hydranths were similar to each other as well as to those of extracts of mature hydranths, except that cystine, methionine, and phenylalanine were not detected. Thus of the twenty-one free amino acids present in mature hydranths, eighteen were detected in hydrocauli and regenerated hydranths.

The free amino acid composition of alcohol extracts of *T. spectabilis* hydranths was similar to that of hydranth extracts of *T. crocea* except that phenylalanine and histidine were not detected on *T. spectabilis* chromatograms. Although similar in free amino acid composition to alcohol extracts of *T. crocea* hydrocauli, the extracts of *T. spectabilis* hydrocauli lacked—in addition to cystine, methionine and phenylalanine—asparagine, histidine, hydroxyproline, and proline. The qualitative differences between the free amino acids of hydranths and hydrocauli of the two species may be significant since the alcohol extracts had similar nitrogen contents.

Seventeen unidentified spots which did not correspond to any simple amino acid spots were detected on chromatograms developed with ninhydrin and other reagents. Table I shows eleven unidentified ninhydrin positive substances detected on two-dimensional chromatograms of *T. crocea* tissues. The patterns of crushed tissues were

¹ Part of a dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Florida State University. Aided by a National Science Foundation Predoctoral Fellowship and a National Science Foundation Grant to Dr. C. B. METZ. Contribution number 122 from the Oceanographic Institute, Florida State University.

² I. FAULHABER and P. TARDENT, Rev. suisse Zool. 66, 295 (1959).

³ J. AWAPARA, Arch. Biochem. 19, 172 (1948).

⁴ A. A. BUZZATI-TRAVERSO, Proc. nat. Acad. Sci., Wash. 39, 367 (1953).

⁵ G. TOENNIES and J. J. KOLB, Analyt. Chem. 23, 823 (1951).

⁶ S. ARONOFF, Techniques of Radiochemistry (Iowa State College Press, Ames, Iowa 1956).

⁷ A. SAIFER and I. ORESKES, Science 119, 124 (1954).

⁸ R. J. BLOCK, J. Dairy Sci. 34, 1 (1959).

⁹ J. B. JEPSON and I. SMITH, Nature 172, 1100 (1953).

Tab. I. Unidentified ninhydrin positive substances on two-dimensional chromatograms of *Tubularia crocea*

Spot	R_f		Crushed			Alcohol Extract	
	a	b	Hydro-caulus	Mature Hydranth	Regen. Hydranth	Hydro-caulus	Mature Hydranth
1	0.34	0.92	+	+	+	+	+
2	0.54	0.94	+	+	—	—	—
3	0.09	0.10	+	+	+	+	+
4	0.07	0.05	+	+	+	—	—
5	0.05	0.03	+	+	+	—	—
6	0.03	0.02	+	+	+	—	—
7	0.08	0.90	—	—	—	—	+
8	0.06	0.79	—	—	—	+	+
9	0.06	0.67	—	—	—	—	+
10	0.25	0.11	—	—	—	+	+
11	0.03	0.07	+	—	+	—	—

^a R_f in butanol: acetic acid: water. ^b R_f in 80% phenol.

Discussion. The detection in the present study of more free amino acids in tubularian tissues and few if any qualitative differences in the distribution of these amino acids as compared to the number reported previously² is probably due to the larger amounts of material chromatographed in the present study. The present results, however, do not negate the possibility of morphogenetically significant quantitative differences in the free amino acid composition of mature and newly regenerated hydranths and hydrocauli as well as hydrocaulus tissues during hydranth regeneration.

The qualitative distribution patterns of several unidentified substances suggest that their identity and fate during hydranth regeneration should be examined.

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Oceanographic Institute, Florida State University, Tallahassee (Florida), February 15, 1960.

Tab. II. Non-amino acid substances of alcohol extracts of *T. crocea* and *T. spectabilis* with reagents for imidazoles (modified Pauly's tests), guanidines (Sakaguchi reaction), -SS- bonds (nitroprusside test), SH groups (nitroprusside test), the hydroxyamino acids, serine, and threonine (Nessler periodate test)

Spot	R_f a	Character of Spot						Presence or Absence of Spot			
		Ninhydrin	Imidazoles	Guanidine	-SS-	SH	Hydroxy-amino acid	<i>T. crocea</i>		<i>T. spectabilis</i>	
								Hydranth	Hydro-caulus	Hydranth	Hydro-caulus
1	0.34	+	—	+	—	+	+	+	+	+	—
3	0.09	+	+	+	+	—	+	+	+	+	—
12	0.12	—	—	—	+	—	—	+	+	+	+
13	0.22	—	—	—	—	+	—	+	—	+	—
14	0.28–0.32	—	+	—	—	—	—	+	+	+	+
15	0.44	—	—	+	—	—	—	+	—	+	—
16	0.66	—	+	—	—	—	—	+	+	+	—
17	0.88	—	+	—	—	—	—	—	+	—	+

^a R_f in butanol: acetic acid: water.

similar except that spot 11 was not present on mature hydranth chromatograms and spot 2 was not present on regenerated hydranth chromatograms. The absence of spots 2, 4, 5, 6, and 11 from chromatograms of hydrocaulus and hydranth alcohol extracts is probably due to alcohol precipitation of substances associated with these spots. Spots 7, 8, 9, and 10 appeared on chromatograms of hydranth extracts; but only spots 8 and 10 were detected on chromatograms of hydrocaulus extracts.

On treating one-dimensional chromatograms of alcohol extracts with reagents for functional groups on certain amino acids, spots 1 and 3 were developed with several reagents (Table II). As seen in Table II of the six additional spots which were ninhydrin negative, spots 13 and 15 were detected only in hydranth extracts of the two species while spot 17 was detected only in stem extracts of the two species. In addition spots 1 and 3 were not detected in extracts of *T. spectabilis* hydrocauli.

Résumé

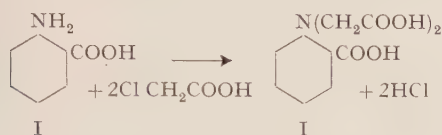
La composition de l'amine acide libre dans les deux espèces de *Tubularia* a été examinée au moyen de la chromatographie. Les tissus d'hydranthe de *T. crocea* avaient 21 amines acides libres dont 18 ont été identifiées dans les hydrocauli et dans les hydranthes régénérés de *T. crocea*, 19 dans les hydranthes de *T. spectabilis* et 14 dans les hydrocauli de *T. spectabilis*. La différence principale entre les hydranthes et les hydrocauli réside dans la distribution de certaines taches non-identifiées qui apparaissent dans les chromatogrammes développés avec la ninhydrine et les réactifs pour les groupes des imidazoles, guanidines, ou sulfhydryles.

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The Accumulation of 3-Iodo N,N, Diacet-6 Aminobenzoic Acid (IDAB) in Growth Areas of Bone¹

SCHORR *et al.*^{2,3} found that new bone formation in malignant bone conditions, osteolytic and osteoplastic, are stained selectively *in vivo* by alizarin sulphionate. After experiments applying I¹³¹ labelled 4-iodo alizarine had failed due to the fast deiodisation of this compound⁴, the behaviour *in vivo* of 3-iodo N, N diacet-6-aminobenzoic acid labelled with iodine 131 was investigated. This reagent, which probably acts as a tridentate chelating agent, gave positive results, showing superior affinity for new bone formations, as compared to iodo alizarin sulphonic acid.

Experimental. 3 iodo N,N, diacet-6-aminobenzoic acid (IDAB) labelled with I¹³¹, was synthesized by condensation of 4-iodo anthranilic acid with chloroacetic acid.



The labelled IDAB had a specific activity of 1 microcurie/mg and it was used until the activity dropped to one tenth of this value. 0.2, 1.0, and 5% solutions of IDAB at pH 6–7.5 were injected intraperitoneally to young rats (average weight 100 g), which were subsequently sacrificed at predetermined intervals. The specific activity of the fresh tissues was determined and expressed as counts/min/g tissue. The *relative specific activity* is defined as the ratio of the specific activity of a given tissue to that of blood. The % of injected dose was calculated from the activity of the injected dose compared to the activities found in tissues, counted under the same conditions and corrected for the decay of iodine 131. Doses over 10 mg were injected either in a single injection or by repeated injections of 10 mg each, at 2 h intervals. No toxic effects were observed even after doses of 200 mg/rat. The callus in rats and in rabbit were produced by deliberate fracture of the tibia. In the *in vivo* tracing experiment, a dose of 500 microcuries of labelled IDAB in 2 ml of 1% solution was injected to a rabbit (1200 g), the tibia of which was fractured five days before the IDAB administration. The legs of the rabbit were scanned with a medical type collimated scintillation counter.

Results and Discussion. Table I presents the concentrations of IDAB in blood, epiphyses, and in the thyroid gland, related to the injected dose. From this Table the fate of the injected drug may be seen. The data are calculated assuming that the average 100 g rat has 5.5 g of blood, 2.5 g of epiphyses, and 10 mg of thyroid tissue. The specific activity found in other tissues, e.g. muscle and liver, did not differ appreciably from that found in blood.

The biological half-lives for the retention of the I¹³¹ activity in rats were derived graphically. It was found that IDAB is cleared from blood in the first few hours, with a biological half-life of 2 h. Later the excretion rate is reduced, and between 8–64 h after injection, a biological half-life of 20 h was observed.

The concentration of IDAB in bone drops at a much slower rate, namely with an estimated biological half-life of over 100 h. The difference between the rate of clearance of IDAB from blood and from bone, results in a rise of the relative concentration of IDAB in bone as compared to that in blood.

Tab. I. The Fraction of the Injected Dose Retained in Blood, Thyroid, and Epiphyses after Injection of 10 mg IDAB (% of injected dose).

Time (h)	0.5	2	4	8	16	24	36	64
Blood	7.3	2.0	0.59	0.25	0.13	0.14	0.073	0.036
Thyroid	0.087	0.048	0.055	0.092	0.058	0.037	0.028	0.045
Bone	2.7	1.9	0.8	0.47	0.45	0.59	0.41	0.38

Tab. II. The Effect of Dose and Rate of Administration on the Relative Concentrations and on the Percentage of Injected Dose Accumulated in 1 g of Epiphyses. (Rats sacrificed 32–36 h after last injection)

Single Dose	Repeated Doses	Relative Spec. conc.	% of injected dose
10 mg	—	11.7	0.16
—	5 × 5 mg	5.0	0.12
—	4 × 10 mg	8.7	0.14
50 mg	—	12.5	0.16
—	4 × 50 mg	4.9	0.15

It was found that various bone tissues show different affinity for IDAB; epiphyses, skull, and callus formation accumulate IDAB to a higher extent than other bones. The differences between the relative concentrations in the various bone tissues diminishes with time.

The effect of dose of IDAB and its rate of administration is demonstrated in Table II.

It may be concluded that whatever dose of IDAB is administered to rats, disregarding rate of administration, we found about 0.15% of the injected dose/g of average bone tissue, with local concentrations two fold higher in areas of rapidly growing bone. Taking the rate of excretion of IDAB into consideration, these values suggested its application for the detection *in vivo* of growing parts of bone. In a preliminary experiment, a callus formation in the tibia of a rabbit was detectable by *in vivo* scanning. Ten days after administration of IDAB, a local activity 6 fold higher than in neighbouring bones was demonstrated.

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Résumé

IDAB marqué avec Iode 131 était administré aux rats pour déterminer sa distribution entre les tissus des rats. On trouve que IDAB est accumulé selectivement par les parties croissantes des os. Deux ou trois jours après l'injection, l'activité spécifique des os était vingt fois plus grande que celle qui se trouve dans le sang.

¹ This investigation was supported by the James Picker Foundation on recommendation of the Committee on Radiology, National Academy of Sciences National Research Council, Washington.

² S. SCHORR and J. AVIAD, *Harefuah* 56, 53 (1959).

³ S. SCHORR, J. AVIAD, and A. LAUFER, *Radiology* 73, 410 (1959)

⁴ S. SCHORR, J. AVIAD, M. ANBAR, and R. REIN, *Amer. J. Roentgenol.*, in press.

Stabilisierung der oxydativen
Phosphorylierung isolierter Mitochondrien
durch artspezifische und
artunspezifische Serumproteine

Bei der Untersuchung der oxydativen Phosphorylierung isolierter Mitochondrien im Warburg-Versuch liegen die gemessenen P/O-Quotienten oft erheblich unter den theoretischen Erwartungswerten. Hauptgrund dafür ist die Labilität des transphosphorylierenden Systems, das schon während der Isolierung der Mitochondrien aus tierischen Geweben geschädigt und dessen volle Funktion unter den meisten Versuchsbedingungen nur kurzfristig aufrechterhalten werden kann.

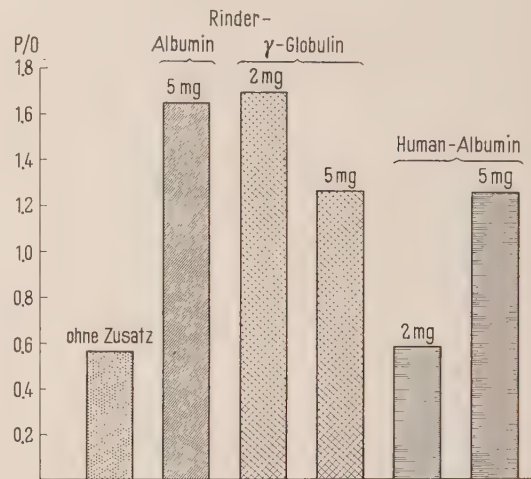
Bei der systematischen Prüfung experimenteller Faktoren, die eine Aufrechterhaltung der Phosphorylierungskapazität über grössere Zeiträume gewährleisten sollten, untersuchten wir auch den Einfluss des Zusatzes verschiedener Eiweisskörper zum Reaktionsmedium, da in der Literatur ein stabilisierender Effekt von Serumalbumin beschrieben worden war¹⁻⁴. Die genannten Untersucher verwendeten allerdings meist artfremdes Serumalbumin (Rinderalbumin) bei Ansätzen mit Mitochondrien aus Ratten- und Mäuseorganen, teilweise auch von Tauben⁵ und sogar Insekten⁶. In unseren Versuchen mit Rinderlebermitochondrien setzten wir sowohl art Eigenes (Rinderserumalbumin und - γ -Globulin) als auch artfremdes Eiweiss (Humanalbumin) den Ansätzen zu.

Methodik. Isolierung der Mitochondrien und Messtechnik wie früher beschrieben⁷. Substrat Bernsteinsäure. Endkonzentrationen im 2,4-ml-Ansatz (einschliesslich 0,4 ml Mitochondriensuspension): Phosphatpuffer 0,012 M/pH 7,2; KCl 0,0215 M; NaF 0,016 M; Natriumsuccinat 0,0053 M; ATP 0,002 M; MgSO₄ 0,006 M; Triäthanolaminhydrochloridpuffer 0,028 M/pH 7,5; Glucose 0,026 M; Hexokinase (SIGMA Typ III) 3 mg; CO₂-Absorption durch 0,2 ml 10%ige KOH im Zentralgefäss. Eiweisszusätze vgl. Abbildung und Tabelle. Die Eiweisskörper stammten von den Behring-Werken. Phosphatbestimmung nach GARBADE. Insgesamt wurden 124 Versuche durchgeführt.

Ergebnisse. Wie die Tabelle zeigt, steigert Zusatz von Rinderalbumin auch den P/O-Quotienten frisch hergestellter Mitochondrien. Diese Steigerung wird hauptsächlich durch Vergrösserung der Phosphataufnahme bewirkt und ist von der eingesetzten Albuminmenge abhängig. Bei 3–5 mg Albumin pro Ansatz liegt die Phosphataufnahme konstant um 31%, die Sauerstoffaufnahme um etwa 10% und der P/O-Quotient dadurch rund 16% gegenüber dem Ausgangswert ohne Albumin höher. Das bedeutet, dass die Mitochondrien trotz schonender Isolierungstechnik bereits während der Aufarbeitungszeit (ca. 2 h) in ihrer Phosphorylierungsfunktion geschädigt werden. Durch ausreichenden Albuminzusatz wird dann ein P/O-Quotient von im Mittel 1,95 erzielt, der den theoretischen Erwartungswert von 2,00 weitgehend erreicht.

Die Senkung der Phosphataufnahme wird noch deutlicher, wenn man die Mitochondriensuspensionen ohne Substratzusatz 3 h auf Eis stehen lässt. Sie kann 10 bis 60% des Ausgangswertes betragen, während die Sauerstoffaufnahme um höchstens 10% sinkt. Dieses unterschiedliche Verhalten hängt bei gleichbleibender Isolierungs- und Messtechnik unter anderem vom allgemeinen Gesundheits- und Ernährungszustand der Rinder, speziell vom Zustand der Lebern ab und wird auch dadurch beeinflusst, dass zwischen Entnahme der Lebern und Beginn der Aufarbeitung naturgemäss mehr Zeit vergeht als zum Beispiel der Herstellung von Mitochondrien aus Rattenlebern.

Auch bei diesen Mitochondriensuspensionen wird durch Albuminzusatz die Phosphataufnahme wieder angehoben, die P/O-Werte erreichen fast die der Frischsuspension in den Ansätzen mit Albumin und liegen sogar höher als die P/O-Quotienten der albuminfreien Frischansätze (vgl. Tabelle). Es scheint daher zweckmässig, bei Warburg-Untersuchungen von Mitochondrien routinemässig den Ansätzen Albumin zuzufügen.



Einfluss verschiedener Eiweissarten und -konzentrationen auf den P/O-Quotienten gealterter Rinderlebermitochondrien.

Wirkung von Rinderalbumin. Messwerte nach 25 min

Albumin-Zusatz	Aufnahmen in μg -Atomen				P/O-Quotient	
	Phosphat		Sauerstoff		t_0	t_3
	t_0	t_3	t_0	t_3		
Null	9.68	8.29	5.89	5.55	1.64	1.49
1 mg	10.32	9.13	5.89	5.55	1.75	1.65
2 mg	11.65	10.29	6.21	5.90	1.88	1.74
3 mg	12.67	11.40	6.50	6.20	1.95	1.84
4 mg	12.67	11.40	6.50	6.20	1.95	1.84
5 mg	12.67	11.40	6.50	6.20	1.95	1.84

t_0 = Messung sofort anschliessend an den Isolierungsvorgang.

t_3 = Messung nach 3stündiger Aufbewahrung der gleichen Mitochondriensuspensionen auf Eis.

Albumin wurde dem Reaktionsansatz, nicht direkt der Mitochondriensuspension zugesetzt. Mitochondrien-N im Mittel 3,7 mg/ml Suspension. Mittelwerte aus 60 Versuchen.

- 1 S. E. LEWIS and E. C. SLATER, *Biochem. J.* **58**, 207 (1954).
- 2 M. I. WATANABE and C. M. WILLIAMS, *J. gen. Physiol.* **37**, 71 (1953/54).
- 3 H. STERN and S. TIMONEN, *Exp. Cell Res.* **9**, 101 (1955).
- 4 M. E. PULLMAN, E. RACKER, *Science* **123**, 1105 (1956).
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- 6 B. SACKTOR and D. COCHRAN, *J. Amer. chem. Soc.* **78**, 3227 (1956).
- 7 G. LAUDAHN, *Z. phys. Chem.* **314**, 95 (1959).
- 8 B. D. POLIS and H. W. SHUMKLER, *J. biol. Chem.* **227**, 419 (1957).
- 9 *Humanalbumin*, Übersichtsbrochure (Behring-Werke, 1959).

Die stabilisierende Wirkung von 5 mg Rinderalbumin/Ansatz kann durch 2 mg Rinder- γ -Globulin ebenfalls erzielt werden. Bei Zusatz grösserer Mengen γ -Globulin (bis 5 mg) wird bei gleichbleibender O_2 -Aufnahme die Phosphataufnahme wieder geringer und demgemäss sinken die P/O-Quotienten (Abb.). Humanalbumin hat einen wesentlich geringeren Effekt als Rinderalbumin: 1–2 mg haben keine Wirkung, 3–5 mg steigern zwar die Phosphataufnahme, aber nicht in dem Masse wie Rinderalbumin. Die Untersuchungsergebnisse beweisen damit eine überlegene Schutzwirkung des arteigenen Albumins. Unter Berücksichtigung der pro Warburg-Ansatz eingesetzten Mitochondrienmenge (als Mitochondrien-N) ist im Querschnitt aller Versuche ein optimaler Effekt bei Zugabe von Rinderalbumin im Verhältnis 3 mg/mg Mitochondrien-N zu beobachten.

Nach heutigen Erkenntnissen beruht die Albuminwirkung auf der Bindung des Chromoproteids «Mitochrom», das von alterierten Mitochondrien freigesetzt wird und die oxydative Phosphorylierung hemmt⁸. Die molekulare Konfiguration des Albumins soll bei der Bindung des Mitochrom eine geringere Rolle spielen als spezifische Endgruppen in den Peptidketten, da partiell denaturiertes Albumin seine positive Wirkung auf die Phosphorylierung behält. Da die Artspezifität ebenfalls in den Hauptvalenzen der Peptidketten verankert ist⁹, kommt dadurch möglicherweise auch die bessere Wirkung arteigenen Albumins zustande.

G. LAUDAHN

Hauptlabor des Städtischen Krankenhauses Berlin-Wilmersdorf, 4. März 1960.

Summary

Addition of 3 mg bovine serum albumin/mg mitochondrial-N enhances and stabilizes the phosphorylation capacity of isolated beef liver mitochondria. Albumin reduces the fluctuation of the resulting P/O-rates and brings about a better reproducibility of the entire method. The same effect is produced by addition of bovine γ -globulin in half the concentration of albumin, while the effect of heterologous proteins (e. g. human albumin) is inferior.

Secretory Cycle and Disappearance of the Prothoracic Glands in *Tenebrio molitor* L. (Coleoptera: Tenebrionidae)

Cyclic changes have been observed in the prothoracic glands of several larval instars of *Tenebrio molitor*. In each instar, the gland is much attenuated shortly after moulting, the web of cells stretching between the tracheae is inconspicuous and the layer of cells normally spread over the dorsal tracheal trunk is observed with difficulty¹. The cells have hyaline cytoplasm with very few and small vacuoles and a few minute granules at the periphery, but without secretion droplets. The nuclei are compact and filled with numerous small chromatin granules.

In the two-week old instar, the gland is considerably developed. The layer of gland cells over the dorsal tracheal trunk is prominent and uniform. The cytoplasm is uniformly granular and minute secretion droplets can be observed in fresh preparations. The nuclei become larger and block-like, elongated, and sometimes lobulated, and chromidial substance is concentrated beneath the nuclear membrane.

In the four-week old instar, 3 or 4 days before the next moult, all the parts of the gland show maximum development and it extends somewhat onto the ventral tracheal trunk also. The cytoplasm has numerous fine granules and sometimes fibrils arranged along the axis of the gland, as reported by ARVY and GABE² in the Ephemeroptera. Secretion droplets are now observed in fresh preparations.

Two days or less before the next moult, the gland is again much reduced, and the sheath over the dorsal tracheal trunk is difficult to discern. The cytoplasm is almost free from granules and secretion droplets, and empty vacuoles are seen. The nucleus is also smaller, thin and elongated and darkly staining.

In the last instar also, similar changes occur. The gland shows maximum development about the time when the larva stops feeding, and about 24 h later, it shows great reduction. The band-like gland assumes the shape of a strand and the elongated nuclei are arranged length-wise in it, but the cells over the dorsal tracheal trunk can still be seen. In the 16 h old pupa, the gland has already lost its discrete nature and the web between the tracheal trunks is disintegrating. Soon after, the gland is represented only by a few cells attached to the dorsal tracheal trunk; and by the time the first traces of eye pigmentation appear, the gland has lost its identity completely.

Thus in each instar of *Tenebrio*, the prothoracic glands undergo maximal development about two days before moulting, when it discharges its secretion to become reduced again. Hence, the critical period occurs about two days before the next moult. In *Anisotarsus*, NÚÑEZ³ described the critical period to occur about 20 h before moulting. In *Dysdercus*, WELLS⁴ found that the volume of nuclear increase at the peak period of the development of the gland is about 5 times its original volume. Such marked increase in nuclear volume does not occur in beetles. In *Rhodnius*⁵ and *Dysdercus*, degeneration of the gland starts about two days after the completion of metamorphosis, but in *Platysamia* WILLIAMS⁶ found that the degeneration commences soon after eye pigmentation has started. Both in *Tenebrio* and *Anisotarsus*, the gland disappears long before metamorphosis is completed, and in *Tenebrio*, it is already disintegrating when eye pigmentation appears, showing that the completion of metamorphosis does not need secretory activity of the prothoracic gland throughout the process. WIGGLESWORTH⁷ suggested that the disappearance of the gland is due to some change (metamorphosis) of the gland itself during the preceding moult, and to some humoral stimulus acting during the last moult. In *Tenebrio*, occurrence of initial changes in the last larval instar, which are similar to those occurring in the earlier ones, indicates that the first factor is not a very important one, while the influences working during the last moult are strong and decisive.

The author is grateful to Prof. O. W. RICHARDS for encouragement and help.

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Department of Zoology, University of Allahabad (India), March 3, 1960.

¹ U. S. SRIVASTAVA, Quart. J. micr. Sci. 100, 51 (1959).

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³ J. A. NÚÑEZ, Biol. Zbl. 73, 602 (1954).

⁴ M. J. WELLS, Quart. J. micr. Sci. 95, 231 (1954).

⁵ V. B. WIGGLESWORTH, J. exp. Biol. 29, 561 (1952).

⁶ C. M. WILLIAMS, Biol. Bull. Wood's Hole 103, 120 (1952).

⁷ V. B. WIGGLESWORTH, J. exp. Biol. 32, 485 (1955).

Zusammenfassung

Bei Tenebrio-Larven werden cyclische Veränderungen der Prothoraxdrüsen beschrieben. Sie bestehen in Vergrößerung der Drüse, verbunden mit Bildung von Körnchen, Fibrillen und Sekrettröpfchen im Cytoplasma. Etwa zwei Tage vor der Häutung wird das Sekret ausgestossen und die Drüse wieder verkleinert. Im letzten Larvenstadium liegt diese kritische Phase 24 h nach Aufhören des Fressens. Die Drüse degeneriert in der Puppe bei Beginn der Augenpigmentierung.

Activation of Pancreatic Trypsinogen by Liver and Kidney Mitochondria

Following upon observations made in our Institute (in publications for the 2nd Italian Congress of Histochemistry, at Florence, 1959) on the inhibition of succinoxidase activity of liver mitochondria by pancreatic granules and mitochondria, I decided to investigate whether such inhibition might be related to a tryptic action of the isolated pancreatic structures on liver mitochondria. For the first time, I was able to demonstrate that, like pancreatic granules, so also pancreatic mitochondria can develop tryptic activity when activated by enterokinase¹.

Afterwards SCHEPOWALNIKOW² demonstrated the existence of enterokinase, several discussions occurred between the various authors about its identity and action. PAVLOV, BAYLISS³, ZUNZ, VERNON, HAMBURGER, STASANO, and others concluded that there is more than one proteolytic enzyme of pancreatic production. VERNON stated that, to activate trypsinogen into trypsin, enterokinase was not indispensable, as the simple addition of trypsin to trypsinogen was enough to catalyse the reaction. Finally KUNITZ⁴⁻⁶ observed that it was possible to obtain activation of trypsinogen by a kinase of Penicillium. NORTHROP and KUNITZ⁷ concluded that reaction trypsinogen-trypsin can be catalysed by the same trypsin, by kinase of Penicillium, and by enterokinase. Considering that such a reaction could perhaps be catalysed by isolated structures of liver, and or of other organs, I considered the action of liver and other organs mitochondria on pancreatic trypsinogen.

Material and Method. For my research I used eight guinea pigs having an average weight of about 300 g each. Immediately after having killed each animal by beheading, I collected its pancreas and the other organs to be examined; after having homogenized each organ in sucrose solution 0.25 M, I made fractionated centrifugation in cold room for 10 min at 2000 g on a first time, and then for 15 min at 10000 g; by this method I obtained isolated mitochondria of the various organs (liver, kidney, muscle, heart, lung, spleen, suprarenals) by 1 g of fresh organ.

In the meantime I homogenized pancreas, and by the method described by NOVELLI⁸ I isolated in sucrose solution 0.88 M granules and mitochondria of pancreas that were suspended for a second time together in distilled water. While mitochondria isolated by 1 g of fresh organ were put into 2 ml of distilled water, a suspension of pancreas was prepared separately and total N₂ was titred by micro-Kjeldahl method. 1 ml of pancreas titred suspension was put with 1 ml of isolated mitochondria in test-tubes containing denaturated hemoglobin, and were incubated for 10 min at 25°C according to the method of

	Milliequivalents of tyrosin × 10 ⁻⁴ per 1 mg of pancreatic N ₂	Tryptic Action
Liver mitochondria + pancreas	550.38 ± 103	Present
Kidney mitochondria + pancreas	284.80 ± 44	Present
Muscle mitochondria + pancreas	—	Absent
Heart mitochondria + pancreas	—	Absent
Suprarenals mitochondria + pancreas	—	Absent
Spleen mitochondria + pancreas	—	Absent
Lung mitochondria + pancreas	—	Absent

ANSON⁹; in the meantime a control was prepared for each test. Complete description of this method has been already reported by myself in another work¹.

Tryptic activity was titrated by Beckman spectrophotometer on the basis of the tyrosin developed, and milliequivalents of tyrosin × 10⁻⁴ were calculated on the basis of 1 mg of pancreatic N₂.

Conclusions. The results of this research permit the conclusion that liver mitochondria contain an activator of pancreatic trypsinogen that acts like the activators previously described, that is by producing the reaction trypsinogen-trypsin. Kidney mitochondria were demonstrated to have the same capacity to activate trypsinogen, although at a lower rate.

As to what concerns the nature of the activator contained by liver and kidney mitochondria, the hypothesis that it could be the same enterokinase appears the most probable, although it still needs specific research for confirmation.

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Istituto di Patologia generale dell'Università di Genova (Italy), February 29, 1960.

Riassunto

Si dimostra la possibilità di catalizzare la trasformazione del tripsinogeno in tripsina mediante aggiunta di mitocondri isolati dal fegato e dal rene; i mitocondri del muscolo, polmone, cuore, surrene, milza non catalizzano tale reazione. La significatività dei risultati viene accertata calcolando il «t» di Fisher.

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² N. P. SCHEPOWALNIKOW, Malys Jb. 29, 378 (1899).

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⁷ J. H. NORTHROP, M. KUNITZ, and R. M. HERRIOTT, *Crystalline Enzymes* (Columbia Univ. Press, New York 1959).

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Thiamine and its Mono-, Di-, and Triphosphoric Esters Content of Normal Rat Tissues

Up to the present, no methods have been described for the separation and determination of Thiamine¹ and its phosphoric esters in animal tissues (particularly TTP recently detected in liver², and in kidneys and brain³ of rats).

Content of T, TMP, TDP, and TTP in some rat tissues (mean ± s. e.)

Compound	Brain (5)		Liver (6)		Heart (5)		Kidney (7)	
	µg/g	%	µg/g	%	µg/g	%	µg/g	%
Thiamine	0.11 ± 0.08	4.4	0.24 ± 0.01	3.5	0.16 ± 0.02	2.3	0.22 ± 0.02	6.2
Thiamine monophosphate	0.30 ± 0.09	11.5	0.66 ± 0.01	9.4	0.41 ± 0.05	5.9	0.35 ± 0.06	9.8
Thiamine diphosphate	2.61 ± 0.15	78.9	6.81 ± 0.44	77.9	7.44 ± 0.44	86.0	3.47 ± 0.13	78.6
Thiamine triphosphate	0.19 ± 0.03	5.0	0.92 ± 0.13	9.0	0.57 ± 0.04	5.6	0.27 ± 0.01	5.2

(): Number of determinations; %:T, as percentage of total T found in the tissue.

On the basis of a previous research⁴, in which the analytical conditions necessary for the chromatographic separation and the estimation of T, TMP, TDP and TTP in pure solutions were described, we have worked out a quantitative method for their estimation in animal tissues. The principle of this method can be summarized as follows:

The tissue is homogenized in cold 5% TCA. The extract, free from proteins, is adjusted to pH 6.7–6.8 with 40% NaOH, and passed through a charcoal column, prepared according to SILIPRANDI and SILIPRANDI⁵. After washing with H₂O, an elution with 60–70 ml of 10% *n*-propanol is carried out. The eluate is concentrated to about 5 ml in a Rinco rotating evaporator at 25–30°C, under vacuum. The concentrate and washings (15 ml), after addition of 0.8 ml 0.1 N HCl, are chromatographed on Dowex 1, × 8, acetate form, column, size 8 × 25 mm, and washed with 10 ml of H₂O. The percolate and washings are collected in a 25 ml volumetric flask and the T and TMP content is estimated by difference before and after Takadiastase digestion (Thiochrome method⁶). The TDP is eluted from the resin column by means of 20 ml of 0.02 M sodium acetate solution in 0.04 M acetic acid. TTP is eluted last with 20 ml of M acetate buffer at pH 4.5. After Takadiastase hydrolysis, the TDP content is determined directly in the eluted solution, and the TTP content after percolation through Amberlite IRC 50, buffered at pH 4.5⁷.

With this method the recovery of the extracted T-compounds is about 95% with good reproducibility. A series of determinations carried out on rat tissues have given the results reported in the Table.

As can be seen, all the tissues examined contain small amounts of TMP and TTP, the biochemical significance of which is still to be elucidated. The organ richest in TTP is the liver, followed by the heart, kidney, and brain. However, by far the most abundant T compound (about 80% of the total T) is TDP.

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Istituto di Fisiologia, Università di Pavia (Italy) and Vister Research Laboratories, Casatenovo (Italy), April 21, 1960.

Riassunto

L'uso di un nuovo metodo cromatografico per la separazione della Tiamina e dei suoi esteri mono, di, e trifosforico nei tessuti animali ha permesso di definire, per la prima volta in termini quantitativi, la presenza del trifosfato nei tessuti stessi. L'organo più ricco di questo estere è il fegato, seguito dal cuore, dal rene e dal cervello nell'ordine.

¹ The following abbreviations have been used: T = Thiamine; TMP = Thiamine monophosphate; TDP = Thiamine diphosphate; TTP = Thiamine triphosphate; TCA = Trichloroacetic acid.
² A. ROSSI-FANELLI, N. SILIPRANDI, and P. FASELLA, *Science* **116**, 711 (1952).
³ H. GREILING and L. KIESOW, *Z. Naturforsch.* **13b**, 251 (1958).
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⁶ Assoc. Vitamin Chemists, *Methods of Vitamin Assay* (Inter. Publ., 2nd Ed., 1951), p. 111.
⁷ E. E. VANNATTA and L. E. HARRIS, *J. Amer. pharm. Ass.* **48**, 34 (1959).

Synthesis at High Pressure and Lattice Constants of Normal Cupric Carbonate

Normal cupric carbonate, CuCO₃, has not previously been prepared, although a number of basic carbonates exist, of which malachite, CuCO₃ · Cu(OH)₂, and azurite, Cu(OH)₂ · 2 CuCO₃, are the best known.

Attempts were made to prepare CuCO₃ by subjecting dry cupric oxide to CO₂-pressures of up to 5000 bars and temperatures ranging from 100°C to 600°C in a hydrostatic bomb which is described elsewhere¹. No reaction took place.

Subsequently a finely ground equimolar mixture of anhydrous sodium carbonate and cupric sulphate was subjected to a pressure of 20 000 bars and a temperature of 550°C in the 'simple squeezer' high-pressure apparatus developed by GRIGGS and KENNEDY². After 1 h under these conditions the sample was quenched at pressure. An X-ray powder diffraction examination of the products showed, in addition to the known patterns of CuSO₄, Na₂CO₃ and Na₂SO₄, a small number of weak lines which could be ascribed to a rhombohedral lattice with nearly the same dimensions as siderite³. The same but weaker

¹ H. HEARD, to be published.
² D. T. GRIGGS and G. C. KENNEDY, *Amer. J. Sci.* **254**, 722 (1956).
³ W. E. SHARP, *Amer. Mineralogist*, **45**, 24 (1960).

lines appeared on examination of the products when precipitated basic cupric carbonate was subjected to 18 000 bars and 550°C in the 'simple squeezer'.

Eventually precipitated basic cupric carbonate was introduced into a low-pressure hydrothermal bomb and subjected to 500 bars total pressure (CO_2 partial pressure = 450 bars, H_2O partial pressure = 50 bars) at 180°C for 36 h. The bulk of the products was well-crystallized malachite, but a small yield of the rhombohedral substance was also obtained.

The yield was better than before, and the diffraction lines were reasonably strong. It proved to be quite simple to subtract the known lines of malachite from the pattern. In only one case was it necessary to estimate the intensity of a line from the earlier patterns.

The X-ray powder diffraction pattern at 25°C was obtained by means of a Norelco high angle recording diffractometer, using $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$) and a Ni filter. The results are given in the Table.

It was found that the presumed CuCO_3 crystallizes in the rhombohedral system. Systematic extinctions appeared to be the same as for calcite, and it is consequently assumed that the space group is also the same, *viz.* $R\bar{3}c$.

The hexagonal unit-cell dimensions at 25°C, obtained by least-squares, are:

$$a_0 = 4.796 \pm 0.005 \text{ \AA} \\ c_0 = 15.48 \pm 0.01 \text{ \AA},$$

yielding an axial ratio

$$c_0/a_0 = 3.227.$$

The dimensions of the corresponding rhombohedral unit-cell are:

$$a_{rh} = 5.856 \text{ \AA} \\ \alpha = 48^\circ 11'.$$

These dimensions are well within the range covered by the other rhombohedral carbonates. While it cannot be stated with absolute certainty that the present compound is actually CuCO_3 , the X-ray evidence is strongly in favour of this being the case.

The stability of this compound is evidently dependent not only on the CO_2 -pressure and the temperature, but also on the partial H_2O -pressure. It would seem that a CO_2 -pressure which is rather high in comparison with the H_2O -pressure is necessary for its stability, and this might explain why it is not found in nature.

Assuming a rhombohedral unit-cell containing 2 molecules, the calculated density of CuCO_3 is 3.99 g/cm³ at 25°C. This value is not dependent on the conditions of formation, as seems to be the case for NiCO_3 ⁴.

Powder data

$d_{\text{obs.}}$ in $\text{\AA}$	$d_{\text{calc.}}$ in $\text{\AA}$	$h\bar{k} \cdot l$	$100I/I_0$
3.654	3.659	01.2	35
2.819	2.831	10.4	100
2.390	2.398	11.0	30
2.161	2.174	11.3	30
1.758	1.757	11.6	25
1.538	1.539	12.2	30
1.451	1.451	10.10	20

The author would like to thank Mr. H. HEARD and Mr. W. E. SHARP, both of the Institute of Geophysics, for their assistance with some of the experimental work.

C. W. F. T. PISTORIUS⁵

Institute of Geophysics, University of California, Los Angeles, March 21, 1960.

Zusammenfassung

In Anwesenheit von Malachit wurde kristallines CuCO_3 durch Behandlung des basischen Karbonates unter 450 bar CO_2 -Druck und bei 180°C gewonnen. Die rhomboedrische Einheitszelle (Raumgruppe $R\bar{3}c$) besitzt die folgenden Dimensionen:

$$a_{rh} = 5,856 \text{ \AA}; \alpha = 48^\circ 11'.$$

⁴ J. GOLDSMITH, oral communication.

⁵ On leave from the National Physical Research Laboratory, Council for Scientific and Industrial Research, Pretoria (Transvaal, Union of South Africa).

Nucleic Acid Content in Mouse Epidermis Separated from Dermis by Different Methods

In various biochemical investigations of the skin, it is essential to obtain data separately for the epidermis and the dermis. This is especially important when pathological processes (e. g. the carcinogenesis) are being studied. In such cases, the interpretation of the data is difficult and often impossible if they refer to the whole skin. That is why different methods for separation of the epidermis from the dermis have been proposed: physical procedures—high vacuum¹; splitting of the epidermis by means of a sharp razor or dermatoms^{2,3}; scraping off the epidermis with a sharp safety-razor⁴ or with a blunt instrument—in the last case after a preliminary loosening of the junction between the epidermis and the corium by means of tight stretching of the skin^{5,6}, or by heating the skin⁷; chemical reagents: unsaturated organic compounds^{8,9}; acids^{7,10,11}; alkali^{7,12}; neutral salts^{10,11}; enzymes: pepsin¹³, trypsin^{14–17}, collagenase, esterase¹⁵.

All methods mentioned above have different advantages and shortcomings, pointed out in the literature cited. It is most probable that for every kind of investigation a different suitable method should be used. The great importance of NA studies in connection with such pathological processes in skin as regeneration, carcinogenesis etc., makes it most desirable to specify which of the suggested methods would be applicable for nucleic acid estimation in epidermis.

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¹⁰ Z. FELSHER, *Proc. Soc. exp. Biol. Med.*, N. Y. **62**, 213 (1946).

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¹⁴ P. B. MEDAWAR, *Nature* **148**, 783 (1941).

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¹⁷ L. BERWICK, *Cancer Res.* **19**, 853 (1959).

Experimental methods. All investigations were carried out on 3-month old Agnes-Bluhm white mice. The hair of the skin was plucked off with a mixture of wax and colophony.

1. *Removal of the epidermis.* (a) *Scraping method.* The incised depilated skin is stretched fur side up and the epidermis scraped off with sharp safety razor. (b) *Heat method*⁷. The skin is placed with corium downwards on a metal plate and heated 2 min at $50^{\circ} \pm 0.5^{\circ}\text{C}$. The epidermis is then removed by means of a blunt scalpel. (c) *Citric acid method.* This procedure, elaborated by us, leads to a spontaneous separation of the epidermis as a whole layer. The sacrificed and depilated animals are injected subcutaneously in the back region with 20 ml of ice cold 2% citric acid, the needle being introduced through the tail in order to avoid the flow out of the solution. The injected mice are kept in the refrigerator at $1\text{--}2^{\circ}\text{C}$ for 2 h and then the epidermis is separated from the dermis as an intact layer. The epidermis separated by this method manifests a considerable O_2 -consumption. (d) *Trypsin method*¹⁵. The incised and depilated skin is incubated at 37°C for 1 h in 0.5% solution of trypsin in phosphate buffer with pH 7.8. The epidermis is then scraped off with a blunt scalpel.

2. *Nucleic acid estimation.* The separated epidermis is immediately placed in ice-cold absolute alcohol. After changing the alcohol, the material is treated twice with ether and dried at 37°C overnight. The estimation of NA was then carried out spectrophotometrically according to our two-wave-length method¹⁸, which eliminates the ultra-violet absorbing contaminants. The results are expressed in mg of phosphorus per 100 g of dried delipidated tissue.

3. *Histological and histochemical controls.* In order to estimate morphologically the degree of separation of the epidermis, as well as the modifying action of the different procedures on the cytochemically detected NA, the separated epidermis and dermis were fixed 40 min in the Helly fixative and paraffin sections were stained with Unna's methylgreen-pyronine.

Results and discussion. The highest values for the NA content are obtained (Table) when the epidermis is removed by scraping or by the heat procedure, both methods giving the same values: 193 mg% RNA-P and 270 mg% DNA-P, the quotient RNA/DNA varying between 0.70 and 0.75. The NA content of epidermis separated by means of citric acid is considerably decreased – 25% for RNA and 33% for DNA, which increases slightly the quotient RNA/DNA to 0.84. Lowest values are obtained by the trypsin method, the RNA content being 31% and the DNA content 43% lower in comparison with the data from the first two methods.

The lower values obtained by the last two methods can be explained by the incomplete separation of the epidermis cells and by the partial extraction of cellular components including NA. As our histological observations have shown, the scraping removes completely the epidermis cells, the quantity of dermis being negligible. The using of a blunt instrument by the heat procedure removes all epidermal cells too, but yields pure epidermis without any traces of the corium. The citric acid separates an intact layer of epidermis, but here and there groups of basal epidermal cells rich in NA remain on the dermis. The most incomplete method is the separation of epidermis with trypsin, a considerable amount of basal cells remaining with the dermis.

The cytochemical detection of NA reveals no visible extraction of NA by all four methods used. Additional biochemical investigations have shown, however, that in citric acid solution a significant amount of substances including NA can be extracted.

Separation method	Replicates	RNA-P ^a	DNA-P ^a	RNA/DNA
Scraping	7	196 $\pm$ 8	262 $\pm$ 11	0.75 $\pm$ 0.03
Heat	14	191 $\pm$ 4	275 $\pm$ 7	0.70 $\pm$ 0.02
Citric acid	6	148 $\pm$ 4	178 $\pm$ 11	0.84 $\pm$ 0.03
Trypsin	7	134 $\pm$ 7	153 $\pm$ 6	0.88 $\pm$ 0.06

^a Expressed as mg of phosphorus per 100 g of dry delipidated epidermis.

Compared with some literature data¹⁹, our values for RNA and DNA in epidermis removed by the heat procedure are high, which indicates that other chemical methods lead to loss of NA too. The higher quotient RNA/DNA (1.44) obtained by the authors cited is due to the contaminants interfering with NA estimation when the classical Schmidt-Thannhauser or Schneider procedures are used¹⁸.

It is obvious from these data that, for NA estimation, only the scraping method especially combined with the heat procedure are suitable for isolation of the epidermis. Separation by chemical reagents or enzymes may influence considerably the NA content of the epidermis.

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Department of Cytology and Cytochemistry, M. Popoff Institute of Biology, Bulgarian Academy of Sciences, Sofia (Bulgaria), March 14, 1960.

Résumé

Des études comparatives ont été effectuées sur le contenu en AN de l'épiderme de souris blanches séparé du derme par quatre méthodes différentes. Ce n'est que la méthode mécanique (scraping method) et la méthode à chaud de BAUMBERGER *et al.*⁷, qui se sont montrées convenables pour les déterminations quantitatives des AN.

¹⁸ R. TSANEV and G. G. MARKOV, Biochim. biophys. Acta, in press (1960).

¹⁹ M. HOLLÓ and SZ. ZLATAROV, Z. Krebsforsch. 60, 624 (1955).

Demonstration of Aliinase in a Protein Preparation from Onion

The recent identification of the major sulfur-containing volatiles of macerated onion as alkyl disulfides¹ plus the observation by VILKKI² that ammonia and pyruvate are released from an amino acid isolated from onion in the presence of onion juice suggests the presence in onions of an enzyme similar to the garlic alliinase of STOLL and SEEBECK³ capable of hydrolyzing S-alkyl-L-cysteine sulfoxides to the corresponding alkyl esters of alkyl thio-sulfonic acids, ammonia, and pyruvate. Recently VIRTANEN and MATIKKALA⁴ have isolated from onion the sulfoxides of both S-methyl and S-propyl cysteine.

¹ J. F. CARSON and F. F. WONG, in press (1960).
² P. VILKKI, Suomen Kemistilehti 27, 21 (1954).
³ A. STOLL and E. SEEBECK, Adv. Enzymol. 11, 377 (1951).
⁴ A. I. VIRTANEN and E. J. MATIKKALA, Acta chem. Scand. 13, 1898 (1959).

Although garlic alliinase was first prepared 11 years ago by STOLL and SEEBECK, no description of its isolation from the related species, onion, has previously been reported. The present paper forwards evidence to support the occurrence of such an enzyme in a soluble protein fraction of onions which was prepared as an acetone powder from an 0.75-saturated ammonium sulfate-precipitated fraction obtained from the supernate of the homogenate of peeled chopped onions. The homogenate was prepared by blending for 30 s 250 g of tissue with 150 ml 0.3 M sucrose in 0.2 M phosphate buffer, pH 6.8, at 4°C.

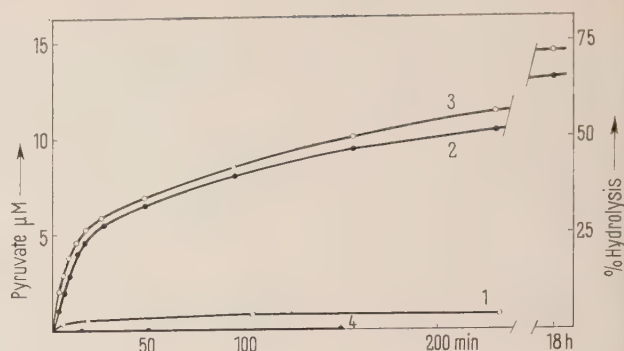
The course of enzymatic production of pyruvate from a synthetic preparation of ($\pm$)-S-propyl-L-cysteine sulfoxide is shown in the Figure. At pH 8.4 in the presence of pyrophosphate buffer and pyridoxal phosphate (conditions for optimal activity), the reaction proceeds rapidly for the first 10 min and 20% hydrolysis to pyruvate. The reaction then proceeds at a slower rate to about 70% in 18 h. The Michaelis constant for this substrate was found to be equal to 5×10^{-3} M. This biphasic mode of the course of the reaction is probably due to the observed difference in the rates of hydrolysis between the (+) and (–) forms of the substrate. The (+) form of S-propyl-L-cysteine is also hydrolyzed faster than (+)-S-methyl and ($\pm$)-S-allyl cysteine sulfoxide. Neither the corresponding S-alkyl-L-cysteines nor cycloalliin⁵ are hydrolyzed. At pH 4.9, the reaction proceeds slowly and terminates at about 4% hydrolysis, thus illustrating the instability and low activity of this enzyme at this pH.

The reaction mixture ('Experimental' 9.1 ml) at pH 8.4 and 37°C in 0.07 M pyrophosphate contained 200 μ M of amino acid (see Fig.), 5 μ M of pyridoxal phosphate, and 18.1 mg of dialyzed enzyme protein nitrogen in a closed Thunberg tube containing 1.4 ml of 1 N HCl in the side arm. The reaction was terminated by addition of the acid in the side arm, centrifuged and stored at –26°C. 'Blank' was identical with 'Experimental' except that amino acid added after addition of HCl. Amino acid was determined by the methods of KORNBERG and PATEY⁶ and ROSEN⁷, pyruvate by the method of KACHMAR and BOYER⁸; ammonia by the method of BROWN *et al.*⁹; thiosulfinate by the method of CARSON and WONG¹⁰, using a pure sample (infrared) of the propyl ester of propylthiosulfinate for standardization. Benzene extract of enzyme digest was used for analysis after removal of benzene by distillation *in vacuo*. (Pyruvic acid was identified by the infrared spectrum of its 2,4-dinitrophenylhydrazone.)

These results (see Table) demonstrate that both ammonia and pyruvate, produced as a result of enzyme action, correspond within experimental error to the decrease in the amount of amino acid. Thiosulfinate as measured by the method of CARSON and WONG¹⁰ appeared in amounts less than half of those expected. This indicates losses due to instability during the reaction (this method does not detect thiosulfonates), during the preparation of the sample for analysis and/or the production of other sulfur-containing substances from the presumed primary product of reaction, propyl thiosulfenic acid³.

The enzyme thus appears to be similar to garlic alliinase in its specificity towards S-alkyl-L-cysteine sulfoxides, its lability³, and in its requirement for pyridoxal phosphate for maximal activity¹¹. It differs from the garlic enzyme in its pH optimum and its optimal action on (+)-S-propyl-L-cysteine sulfoxide. The garlic enzyme has been reported to act optimally on (+)-S-allyl-L-cysteine sulfoxide, over a broad pH range extending from pH 5 to pH 8³. The lack of action on S-substituted cystine derivatives differentiates it sharply from the C-S lyase of *Albizia lophanta*¹².

We wish to thank G. F. BAILEY and E. GONG of this laboratory for the infrared determinations.



Production of pyruvate from S-propyl-L-cysteine sulfoxide by a protein preparation from onions. The enzyme reaction, carried out in 1 ml at 37°C, contained the following: curve 1, 20 μ M of ($\pm$)-S-propyl-L-cysteine sulfoxide ($\alpha_D = -6.9$, $c = 1$) and 1.55 mg of enzyme protein nitrogen at pH 4.9; curve 2, as in curve 1 + 70 μ M pyrophosphate buffer (final pH = 8.4); curve 3, as in curve 4 + 0.05 μ M of pyridoxal phosphate; curve 4, as in curve 3, except boiled enzyme used. Pyruvate determined according to method of KACHMAR and BOYER⁸

Stoichiometry of Onion Alliinase Action

Substance	Blank μ M	Experim. μ M	Change μ M
Amino Acid	200	126	–74
Pyruvic Acid	2	75	+73
Ammonia	6	74	+68
Thiosulfinate	0	34	+34

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Western Regional Research Laboratory, Albany (California), A Laboratory of the Western Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, March 14, 1960.

Zusammenfassung

Eine proteinhaltige Darstellung aus Zwiebeln enthält ein Enzym, welches stöchiometrisch S-Alkyl-L-cystein-sulfoxide in Brenztraubensäure und Ammoniak spaltet. Daneben entsteht, nicht-stöchiometrisch, ein benzollöslicher, lauchartig riechender, mit N-Äthylmaleinimid reagierender Stoff, wahrscheinlich das Alkylthiosulfinsäure-alkylester. Das Enzym wirkt optimal auf (+)-S-Propyl-L-cysteinsulfoxid bei pH 8,4 in Gegenwart von Pyridoxal-phosphat.

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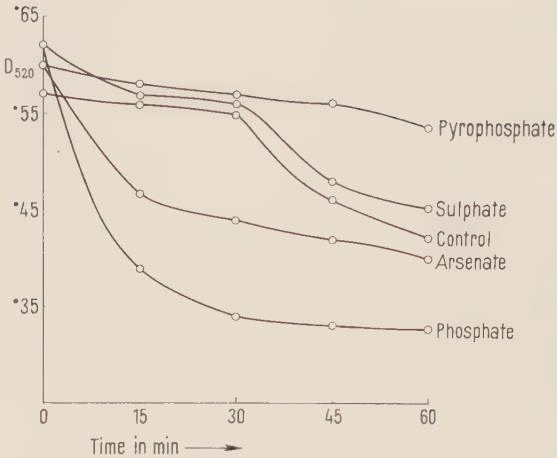
The Correlation between Anion-Induced Mitochondrial Swelling and Glutaminase I Activity of Guinea Pig Liver

In a previous communication¹, we reported the conditions necessary for phosphate activation of glutaminase I of guinea pig liver mitochondria and suggested the possibility of a correlation between anion-induced swelling and the observed glutaminase I activity of mitochondria. Evidence is presented here in favour of such a possible correlation.

Methods. Mitochondria was prepared in 0.44 M sucrose according to the method of SCHNEIDER² and washed thrice with ice-cold sucrose. The basic medium of incubation was 0.44 M sucrose containing 0.05 M Veronal buffer pH 7.2. Enzyme activity was determined by measuring the ammonia formed as reported previously¹, while the corresponding swelling was followed by reading the changes in absorbancy at 520 mμ. Mitochondria equivalent to about 75 mg of wet tissues were taken in all cases and added last to the systems within 15 min of preparation. All additions were brought beforehand to pH 7.2. Ageing of mitochondria was made by preincubation for 20 min at 40°C.

Results. Among the anions tested (0.2 M), only phosphate and arsenate produced marked swelling, whereas sulphate was without any effect; pyrophosphate, on the other hand, inhibited even the spontaneous swelling of mitochondria (Fig.). The corresponding glutaminase activity indicated that phosphate and arsenate activated the enzyme, whereas sulphate produced a very slight increase in activity. On the contrary, pyrophosphate, known as one of the strongest activator of glutaminase I³, completely failed to activate the enzyme even with higher concentration (0.4 M). But when aged and preswollen mitochondria were employed, both pyrophosphate and sulphate produced significant activation of glutaminase I at 37°C (Table I).

Effect of temperature variation on phosphate-induced swelling and glutaminase I activation also showed close correlation between these two functions of the anion. At



Effect of various anions (0.2 M) on swelling of guineapig liver mitochondria suspended in 0.44 M sucrose – 0.05 M Veronal buffer, pH 7.2 at 37°C.

Tab. I. Anion activation of glutaminase I of guineapig liver mitochondria

Anions added (0.2 M)	μg Ammonia formed/h	
	Fresh mitochondria	Aged mitochondria
1. None	7.2	2.0
2. Phosphate	24.0	20.5
3. Arsenate	16.2	18.5
4. Sulphate	9.6	12.0
5. Pyrophosphate	6.8	16.5

All incubations were carried out with L-glutamine ($5 \times 10^{-3} M$), at pH 7.2 and temperature 37°C.

Tab. II. Inhibition of phosphate (0.2 M) induced swelling and glutaminase I activation of guineapig liver mitochondria

Inhibitors added	Fresh mitochondria					Aged mitochondria		
	Changes in O.D./h			% inhibition of swelling	μg Ammonia formed/h	Glutaminase activity (relative)	μg Ammonia formed/h	Glutaminase I Activity (relative)
	Initial	Final	Difference					
1. None	0.72	0.385	0.335	Nil	18.5	100	13.0	100.0
2. KCN (0.1 <i>M</i>)	0.72	0.60	0.12	64.2	8.0	43.2	13.5	103.8
3. NaN ₃ (0.2 <i>M</i>)	0.68	0.54	0.14	58.3	2.0	10.8	1.5	11.5
4. Na-Amytal (0.01 <i>M</i>)	0.72	0.57	0.15	56.3	11.0	59.4	13.0	100.0
5. Antimycin A (5 μg)	0.72	0.55	0.17	49.3	12.5	67.5	13.0	100.0
6. Sucrose (0.88 <i>M</i>)	0.67	0.55	0.12	64.2	7.5	40.0	13.0	100.0
7. Sucrose (0.88 <i>M</i>) + 0.4 <i>M</i> PO ₄	0.67	0.39	0.28	16.5	16.0	86.4	—	—

All incubations were carried out with L-glutamine ($5 \times 10^{-3}M$) at pH 7.2 and temperature 27°C.

37°C, 0.2 M phosphate induced both maximum swelling and glutaminase I activation. At 27°C similar results were found with higher phosphate concentrations (0.3 to 0.4 M), but at 15°C neither swelling of mitochondria nor glutaminase I activation was possible with any concentration of phosphate.

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² W. C. SCHNEIDER, in *Manometric Techniques* (Ed. by W. W. Umbriet, R. H. Burris, and J. F. Stauffer, Burgess Publishing Co., Minneapolis 1957), p. 188.
³ J. D. KLINGMAN and P. HANDLER, J. biol. Chem. 232 369 (1958).
⁴ L. ERNSTER and O. LINDBERG, Ann. Rev. Biochem. 20, 26 (1958), (Ed. by V. E. Hall, Ann. Rev. Inc., U.S.A.).

Known antischwelling agents of mitochondria like KCN, Na-azide, amytal, antimycin A, and sucrose (0.88 M) were tested to observe their effects on both glutaminase I and the corresponding swelling. It was observed that both glutaminase I and swelling of fresh mitochondria were inhibited by these substances. However, with aged and preswollen mitochondria, all these inhibitors, except Na-azide, failed to inhibit the glutaminase I activity (Table II), indicating that the inhibition of enzymic activity by the above substances was primarily due to their antischwelling action on fresh mitochondria. Na-azide, however, was inhibitory to both swelling and glutaminase I. Hypertonic sucrose (0.88 M) in fresh mitochondrial preparation prevented swelling as well as glutaminase I activity with the usual 0.2 M phosphate concentration, but on further addition of phosphate to a final concentration of 0.4 M, both these activities reappeared.

That swelling of mitochondria releases some hydrolytic enzymes has been reported previously⁴; but no work has been done with regard to glutaminase I. Data presented above strongly suggest that swelling of mitochondria caused either by the added anions themselves, like phosphate and arsenate, or due to induced ageing or preswollen condition, as with pyrophosphate and sulphate, is a prerequisite condition for the demonstration of anion activation of glutaminase I of guineapig liver mitochondria.

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Indian Institute for Biochemistry and experimental Medicine, Calcutta (India), March 7, 1960.

Zusammenfassung

Die dargelegten Versuche beweisen, dass die durch Anionen (Phosphat und Arsenat) herbeigeführte Anschwellung von Mitochondrien der Meerschweinchenleber eine Vorbedingung ist für die Glutaminase I-Aktivierung. Pyrophosphat, das die Schwellung hemmt, und Sulfat, das sie nicht beeinflusst, aktiviert das Enzym erst nach vorheriger Anschwellung der Mitochondrien.

Distributions of Cholesterol Amongst Liver Subcellular Fractions

Assays for the cholesterol content of various subcellular fractions derived from rat liver homogenates have been reported by a number of investigators¹⁻⁵ and are presented in Table I. In each instance mitochondria were isolated under conditions of centrifugation (18 000–24 000 × g for 10 min) sufficient to sediment microsomes as well.

Mitochondria obtained from rat and mouse liver homogenates by centrifugation at 8500 × g for 10 min, then washed by resuspension and recentrifugation, have been employed in studies of the cholesterol oxidase system⁶⁻⁹. Addition of the microsomal fraction to these '8500 × g' mitochondria, omission of the washing procedure, or isolation of mitochondria under higher centrifugal forces for the same time interval, in each case severely depressed oxidation of added cholesterol —C¹⁴ by the cholesterol oxidase present in the mitochondria. This has been traced in part to the relatively high endogenous content of free cholesterol in the liver microsomal fraction which effectively dilutes any added radioactive cholesterol.

Table II records the relative distribution of liver cholesterol amongst various subcellular fractions of a number of animals. The fractions were isolated according to the

scheme presented in the Figure. Liver specimens were washed three times with 10% w/v sucrose before homogenization in a Potter-Elvehjem homogenizer. The subcellular fractions were digested in warm 30% aq. KOH and the cholesterol extracted with petroleum ether. After the petroleum ether extracts were dried over anhydrous Na₂SO₄ aliquots were analyzed for cholesterol using the method of TRINDER¹⁰.

The consistency of the liver mitochondrial cholesterol content in several animal species is of interest. SCHOTZ, RICE, and ALFIN-SLATER³ found that their preparations of rat liver mitochondria contained 0.20 mg cholesterol. Our preparations, derived from the same volume of liver homogenate (4.0 ml), contained on the average only about 0.13 mg.

Tab. I. Percentage Recovery of Total Cholesterol in Mouse and Rat Liver Fractions

Fraction	Ref. 1 ^a	Ref. 2 ^b	Ref. 3 ^a	Ref. 4 ^a	Ref. 5 ^a
Nuclei	—	—	6.0	0.7	—
Mitochondria	1.3	3.5	13.7	1.2	2.2
Microsomes	—	5.1	—	1.9	—
Supernatant	—	2.8	—	0.3	—

^a Rat. ^b Mouse.

Tab. II. Average Percentage of Total Liver Cholesterol Recovered (No. of experiments in parentheses)

Fraction	Species					
	Mouse ^a (2)	Rat (3)	Chicken (3)	Rabbit (2)	Monkey (1)	Human (1)
Debris and nuclei	39.0	53.7	60.1	58.0	58.7	56.4
Mitochondria	7.8	6.3	7.6	8.1	6.5	6.9
Mitochond. wash	14.9	7.0	7.0	10.9	6.5	6.9
Microsomes	33.5	25.0	15.3	15.1	9.5	10.5
Supernatant	4.8	8.0	10.0	7.9	18.6	19.5

^a Pooled livers of 4 mice used.

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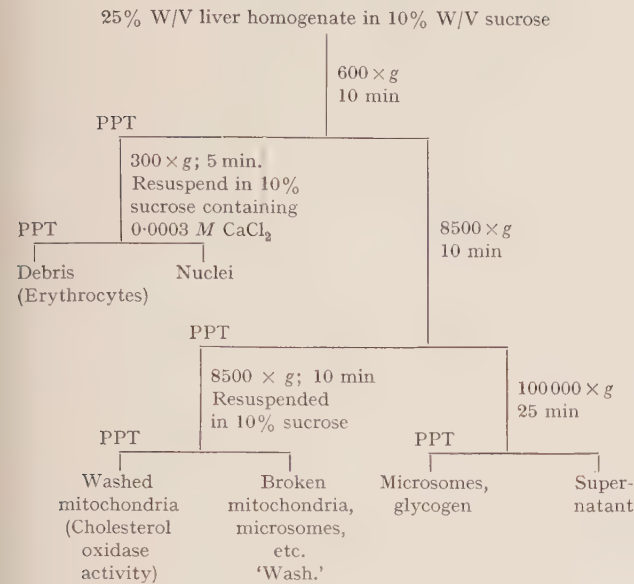
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Scheme of Liver Fractionation



Acknowledgements. We wish to thank Mrs. R. KOLMAN and Mrs. M. COTTRELL for skillful technical assistance. We are grateful to Dr. S. COOPERBAND for making the human liver available to us and to Dr. L. FERRIGAN for the monkey liver.

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Wistar Institute of Anatomy and Biology, Philadelphia (Pennsylvania) and Department of Biochemistry, School of Medicine, University of Pennsylvania, Philadelphia, March 7, 1960.

Zusammenfassung

Der Cholesteringehalt der subzellulären Komponenten von Lebergewebe verschiedener Tierarten wurde untersucht. Die subzellulären Fraktionen bestanden hauptsächlich aus Mitochondrien und waren relativ frei von Mikrosomen.

Transaminasenaktivität nach Methyltestosteron-, Testosteronpropionat-, Chlorpromazin- und Methylthiouracilbehandlung

Eingehendere Untersuchungen über Aktivitätsänderungen der Transaminasen im Serum (SGO-T, SGP-T) liegen vor allem bei Herzinfarkt und bei Lebererkrankungen vor. Anfangs glaubte man, dass bei Lebererkrankungen der Fermentanstieg im Serum von dem Ausmass der Zellschädigung abhängig sei. Später jedoch haben experimentelle Untersuchungen und klinische Beobachtungen gezeigt, dass die Höhe der Serum-Fermentaktivität nicht nur durch die Freisetzung der Fermente aus dem erkrankten Organ beeinflusst wird, sondern auch von der Grösse des Fermentschwundes aus der Blutbahn abhängig ist. Nach AMELUNG *et al.*¹ können an dem Fermentschwund folgende Vorgänge beteiligt sein:

1. intra- oder extravasaler Abbau bzw. Inaktivierung der Transaminasen;

2. Aufnahme der Fermente in aktiver Form durch die Zellen;

3. Ausscheidung der Fermente durch Leber oder Niere. Auf eine Diskrepanz zwischen dem Ausmass der Lebernekrosen und der Aktivitätszunahme der Transaminasen im Blutserum machten schon MOLANDER *et al.*² aufmerksam, die bei Kranken mit cholangiolitischer Zirrhose, wo in der Leber weniger eine Nekrose zu finden war, eine höhere Transaminasenaktivität vorfanden als bei Kranken mit Laenecscher Zirrhose. Einige Arzneimittel (Salvarsan, Methyltestosteron, Paraaminosalicylsäure, Methylthiouracyl, Chlorpromazin, Phenothiazin, Toluylen-diamin, Dinitrophenol, Atophan) können eine cholangiolitische Hepatitis mit Icterus hervorrufen. Grösste Aufmerksamkeit widmet man in letzter Zeit dem Icterus nach der Chlorpromazinbehandlung und seiner Derivate. Der Icterus nach diesem Arzneimittel tritt ungefähr in 1% der Fälle ein, jedoch haben SHAY und SIPLET³ eine Aktivitätszunahme der SGO-T während 1–4wöchiger Therapie bei fast 30% ihrer Kranken festgestellt.

Wir haben uns die Aufgabe gestellt, nicht nur den Einfluss des Chlorpromazins, sondern auch des Methyltestosterons und Methylthiouracils auf die Fermentaktivität der SGO-T und SGP-T zu verfolgen. Wir untersuchten 62 Kranke der Psychiatrischen Abteilung, die mit Chlorpromazin etwas über 2 Wochen behandelt wurden. Eine Aktivitätserhöhung der SGO-T mit einem Maximalwert von 248 Einheiten fanden wir bei 12 Kranken. Die Aktivitätsbestimmung wurde jedoch nicht in allen Fällen von Beginn der Chlorpromazinbehandlung an durchgeführt. Eine 14tägige perorale Methylthiouracilbehandlung (200 bis 400 mg täglich), die bei 6 Kranken angesetzt wurde, rief keinen bemerkenswerten Aktivitätsanstieg hervor. Weitere 40 Kranke bekamen vorwiegend wegen Osteoporose 30 mg Methyltestosteron *per os* täglich. Bei 31 Kranken (77%) wurde nach durchschnittlicher 10tägiger Behandlung ein Anstieg der SGO-T (Maximalwert 275 Einheiten) und der SGP-T (Maximalwert 260 Einheiten) gegenüber dem Ausgangswert vor der Behandlung festgestellt. Die Leberfunktionsteste (Bilirubinämie, Thymoltrübungstest, Takatareaktion und Weltmannkoagulationsband) blieben unverändert. Bei 10 Patienten, die täglich 30 mg Testosteronpropionat i. m. mindestens 14 Tage hindurch erhielten, wurde keine Aktivitätsveränderung der Transaminasen beobachtet. Bei 6 Versuchsratten (3 weibliche und 3 männliche), denen 10 Tage lang täglich 5 mg Methyltestosteron verabreicht wurde, haben wir keine histologischen Veränderungen in der Leber vorgefunden.

An Hand unserer Ergebnisse müssen wir annehmen, dass Methyltestosteron im Vergleich zu Chlorpromazin wesentlich öfters einen Aktivitätsanstieg der Transaminasen hervorruft. Der Aktivitätsanstieg ist beim Methyltestosteron wahrscheinlich durch die Methylgruppe bedingt und braucht nicht immer eine toxische Schädigung der Leber zu bedeuten.

Wir danken Herrn Prof. E. VENCOVSKÝ von der Psychiatrischen Universitätsklinik Plzeň für die freundliche Überlassung des Krankengutes und Herrn Prof. J. VANĚK aus dem Pathologisch-Anatomischen Institut für die Anfertigung der histologischen Präparate.

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Medizinische Universitätsklinik Plzeň (Tschechoslowakei), 3. März 1960.

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Summary

The treatment of 62 patients with chlorpromazine was followed by a rise of SGO-T in the serum in 20% of them. Six other patients were treated with a daily dose of 200 to 400 mg Methylthiouracil for 14 days. No elevation of serum transaminases was observed. The application of a daily dose of 30 mg methyl testosterone to 40 other patients was followed by a rise of SGO-T and SGP-T in 77% of them. The maximal elevation of SGO-T was 275 U. and of SGP-T 260 U., and this was reached during 10 days of therapy on an average. This effect is bound to the methyl group of methyl testosterone, since in 10 patients, treated with the same dose of testosterone propionate, the serum transaminases activity were not influenced.

Einfluss der Wurzeln auf die Trigonellinsynthese bei *Coffea arabica*

In der letzten Zeit wird der Bedeutung der Wurzeln als Syntheseort sekundärer Pflanzenstoffe besondere Aufmerksamkeit gewidmet. Das gilt vor allem für Alkaloide, deren Synthese zum Teil in den Wurzeln lokalisiert werden konnte. Die Biosynthese eines der einfachsten Alkaloide, des Trigonellins, ist bisher nicht aufgeklärt worden.

ZEIJLEMAKER¹ konnte bei Erbsenpflanzen zeigen, dass die N-Methylierung von Nikotinsäure zu Trigonellin auch in abgeschnittenen Sprossen vor sich geht. In dieser vorläufigen Mitteilung möchten wir die Resultate von vergleichenden Versuchen über die Trigonellin-Synthese bei jungen Pflanzen von *Coffea arabica* bekanntgeben. Unter Verwendung von $C^{14}O_2$ sollte markiertes Trigonellin erhalten werden. Es zeigte sich, dass der Einbau von $C^{14}O_2$ in Trigonellin stark abhängig war vom Vorhandensein

Aus der Tabelle mit den Versuchsergebnissen können folgende Schlüsse gezogen werden.

1. Die Variation des absoluten Gehaltes an Trigonellin zeigt, dass in den jungen Blättern (1–2) eine intensive Synthese vor sich geht.

2. Vom 2. bis zum 5. Blatt nimmt der Trigonellin-gehalt ab.

3. Die spezifische Aktivität des Trigonellins ist in den älteren Blättern viel höher als in den jüngeren. Das trifft für alle drei Versuche zu. Die genauere Ursache dieser charakteristischen Altersunterschiede soll durch weitere Untersuchungen aufgeklärt werden.

4. Sowohl Entfernung des Wurzelsystems wie auch die Unterbrechung der Rindenverbindung Sprosswurzel wirken sich in einer beträchtlichen Erhöhung der spezifischen Aktivität des Trigonellins in den Blättern aus.

Es zeigt sich somit, dass die Wurzeln offenbar einen Einfluss auf die Trigonellinsynthese in den Blättern haben. Dieser Einfluss ist wahrscheinlich indirekt. Entfernung der Wurzeln oder Ringelung verhindert den Abtransport der Assimilate in die Wurzeln. Es ist zu erwarten, dass dadurch die spezifische Aktivität von Stoffwechselprodukten, die aus Assimilation entstehen, höher werden kann. Andererseits haben weitere Untersuchungen an diesem Institut Anhaltspunkte dafür gegeben, dass auch Trigonellin aus Blättern abtransportiert werden kann. Wird dieser Transport unterbunden, so könnte eine vorübergehende Erhöhung der spezifischen Aktivität des Trigonellins gegenüber der intakten Kontrolle erwartet werden.

Herrn Prof. Dr. H. WANNER danke ich für die Anregung zu dieser Arbeit. Für die technische Hilfe danke ich Frau M. STAMPELI und Fräulein R. WEGMAN. Diese Arbeit wurde durch ein Stipendium der Rockefeller Foundation ermöglicht. Für materielle Unterstützung danken wir ferner der Schweizerischen Kommission für Atomwissenschaft.

Blattpaar von oben nach unten	Trigonellin								
	intakte Pflanze			Pflanze ohne Wurzeln			Pflanze geringelt		
	mg ^a	% ^b	Spez. Aktivität ^c	mg	%	Spez. Aktivität	mg	%	Spez. Aktivität
1	0,99	0,29	$6,30 \cdot 10^6$	1,21	0,32	$14,39 \cdot 10^6$	0,18	0,13	$12,88 \cdot 10^6$
2	6,05	0,22	$10,27 \cdot 10^6$	5,83	0,27	$15,36 \cdot 10^6$	4,96	0,23	$19,18 \cdot 10^6$
3	2,96	0,12	$2,09 \cdot 10^6$	2,40	0,14	$8,29 \cdot 10^6$	3,71	0,17	$2,05 \cdot 10^6$
4	1,28	0,19	$23,91 \cdot 10^6$	1,48	0,20	$32,07 \cdot 10^6$	2,02	0,23	$49,59 \cdot 10^6$
5	0,22	0,05	$38,36 \cdot 10^6$	0,34	0,07	$48,93 \cdot 10^6$	0,51	0,09	$45,76 \cdot 10^6$
Σ	11,50	—		11,26	—		11,39	—	

^a Gesamtgehalt. ^b Gehalt bezogen auf Frischgewicht. ^c Impulse/min/mM (ipm/mM).

oder Fehlen eines Wurzelsystems. Es wurden 3 Versuchsbedingungen angewendet: 1. Intakte 1jährige Pflanzen mit 5 Blattpaaren über den Kotyledonen. 2. Gleichaltrige Pflanzen mit abgeschnittenem Wurzelsystem (Sprosse in Leitungswasser gestellt). 3. Gleich alte Pflanzen mit 5 mm breiter Ringelung unter den Kotyledonen. Die Pflanzen erhielten unter Belichtung je 200 μC $C^{14}O_2$, die in 6–7 h assimiliert wurden. 24 h nach Versuchsbeginn kamen die Pflanzen zur Analyse. Den Trigonellin-gehalt ermittelten wir durch papierelektrophoretische (Abtrennung der löslichen Kohlehydrate) und papierchromatographische Isolierung der Substanz sowie spektrophotometrische Bestimmung der Konzentration (Absorptionsmaximum 265 m μ). Die Radioaktivität der Proben wurden mit einem Methan-Druckflusszähler in sehr dünner Schicht gemessen. Die Selbstabsorption konnten wir bei einer Schichtdicke von einigen γ/cm^2 vernachlässigen.

K. BLAIM²

Institut für allgemeine Botanik, Universität Zürich,
18. Februar 1960.

Summary

Young plants of *Coffea arabica* were fed 200 μC $C^{14}O_2$ each, the carbon dioxide being absorbed by photosynthesis. The specific activity of the alkaloid trigonelline contained in the leaves was shown to be strongly dependent of leaf age. Excised plants without root system or plants with an interrupted shoot-root connection as regards phloem transport (by girdling the stem below the cotyledons) incorporate more radioactive carbon into the molecule of trigonelline than intact plants.

¹ F. C. J. ZEIJLEMAKER, Acta bot. neerl. 2, 123 (1953).

² Ständige Adresse: Pulawy J.U.N.G. Polen.

The Role of Proconvertin and Stuart Factor in the Inactivation of Tissue Thromboplastin by Serum

It has been found in our previous studies¹ that the tissue thromboplastin inactivating capacity of native serum was significantly increased by partial adsorption with kaolin whereas it was lost by subsequent BaSO₄ adsorption. These results were in good agreement with HJORT's conception², who proved in careful experiments that the participation of factor VII complex—equivalent to his proconvertin reagent—is indispensable for the inactivation of tissue thromboplastin by serum inhibitor. At the same time, it became clear that every system with unadsorbed or partially adsorbed serum is unsuitable for the separate study of the inhibitor proper and of its co-factor contained in the factor VII complex as both these components participating in the process of inactivation react differently on various procedures carried out with serum or in pathologic states. For that reason, a simple system was elaborated for rough quantitative assay of the inhibitor proper, which represented the only variable³. This system was also suitable for the evaluation of the role of proconvertin (i.e. factor VII proper) and Stuart factor in the tissue thromboplastin inactivation by serum inhibitor.

Methods. As substrate, normal freshly prepared, platelet-poor, oxalated plasma was used. Normal native (i.e. untreated) serum was usually 24 h old. The normal adsorbed serum, practically devoid of all factor VII complex activity, was prepared by two-fold adsorption with kaolin (100 mg/ml) and additional adsorption with BaSO₄ (100 mg/ml). In the incubation system, *m*/10 CaCl₂ was used. Acetone-dried human brain extract (200 mg per 10 ml saline) with the activity of 13 ± 1 sec in the Quick test served as substrate for inactivation.

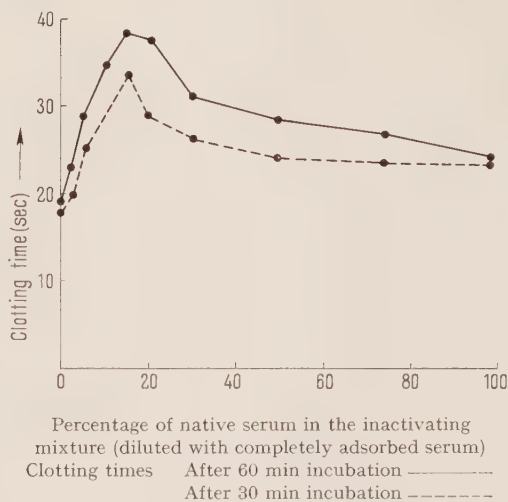
The testing system was similar to that described previously¹, but various inactivating mixtures instead of partially adsorbed serum were examined. In water bath at 37°C, two parts of the mixture tested were added to one part of *m*/10 CaCl₂, and finally one part of tissue thromboplastin was blown in. After 1, 30, and 60 min of incubation, a sample of 0.2 ml was taken and tested for its remaining thromboplastin activity on 0.2 ml substrate plasma. The sera deficient in proconvertin or Stuart factor respectively were kindly supplied to us by Dr. CAËN from Paris in lyophilized form. In addition to the control with normal native serum, the normal dry reconstituted serum was also used.

Results. Native serum contains both factor VII complex and the inhibitor. On gradually increasing the amount of this serum in the inactivating mixture with completely adsorbed serum, the thromboplastin inhibition, expressed by the prolongation of the clotting times after 1 h incubation, rises from zero to a maximum value which is reached at a level of 15% of native serum in the inactivating mixture (Fig.). A further increase above 20% leads, however, to a gradual decline in the thromboplastin inactivation. When the content of native serum is kept constant at 15%, and the 85% of adsorbed serum in the inactivating mixture is serially diluted in advance by veronal acetate buffer, a dilution curve can be obtained for the inhibitor proper. The properties of this inhibitor and its behaviour in various states are presented elsewhere³⁻⁵. The available amount of deficient sera being limited, no detailed quantitative analysis could be performed and these sera were substituted for normal native or dry, reconstituted serum in the inactivating mixture.

When proconvertin-deficient serum was used, no inactivation took place (Table I). As in patients with Stuart factor-deficiency, the content of proconvertin was moderately decreased (60% according to Dr. CAËN), 20% of this reconstituted serum and of normal redissolved serum (second control) was used instead of usual 15% of native serum in the inactivating mixture. In spite of the virtual absence of Stuart factor, no substantial change in the inactivation was found (Table II).

Discussion. Present results confirm again the indispensable presence of proconvertin for tissue thromboplastin inactivation by a natural inhibitor, as was first postulated by HJORT². In his proconvertin reagent, however, the Stuart factor was also present and the possibility of its involvement was disregarded. Nevertheless, the studies of PUDLÁK *et al.*⁶ indicated that Stuart factor may have a special affinity for tissue thromboplastin. In view of our results, a formation of some complex involving both these components does not, however, seem necessary for the action of the inhibitor in contrast with the role of convertin, i.e. proconvertin-calcium-tissue thromboplastin complex which represents most probably the only form inactivable progressively by the natural inhibitor.

This assumption is to some extent in disagreement with observations describing normal inactivation in BaSO₄ adsorbed sera⁷⁻⁹. It seems, however, that no attempt has been made in these studies to determine the possible small residue of proconvertin after adsorption. According to the analysis of HJORT² and of ourselves³, it is very probable that tissue thromboplastin, proconvertin, and inhibitor react in quantitative proportions. If diluted thromboplastin is used for inactivation, a small percentage of proconvertin may be sufficient to 'saturate' the



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² P. F. HJORT, Scand. J. Lab. Invest. 9, Suppl. 27 (1957).

³ F. HEŘMANSKÝ, Studium inaktivace tromboplastinu, Thesis Charles University, Prague (1959).

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⁸ E. DEUTSCH and H. FUCHS, Acta haemat. 20, 97 (1958).

⁹ C. G. BERRY, J. clin. Path. 10, 342 (1957).

amount of thromboplastin present, since about 15% of factor VII complex sufficed for maximal inactivation of undiluted thromboplastin under our experimental conditions.

Tab. I

Inactivating mixture		Clotting times after incubation of		
		1 min	30 min	60 min
Normal native serum 15%	Normal adsorbed serum 85%	12.6"	30.0"	34.1"
Reconstituted normal serum 15%	Normal adsorbed serum 85%	12.7"	29.0"	31.2"
Proconvertin deficient serum 15%	Normal adsorbed serum 85%	15.3"	16.5"	16.6"

Tab. II

Inactivating mixture		Clotting times after incubation of		
		1 min	30 min	60 min
Normal native serum 15%	Normal adsorbed serum 85%	12.6"	26.8"	33.9"
Reconstituted normal serum 20%	Normal adsorbed serum 80%	11.8"	27.4"	34.6"
Stuart factor deficient serum 20%	Normal adsorbed serum 80%	11.6"	26.2"	32.6"

F. HEŘMANSKÝ and J. VÍTEK

Research Laboratory for Hematology and Liver Diseases, 1st Medical Clinic, Charles University, Prague, March 22, 1960.

Résumé

Le rôle de la proconvertine et du facteur Stuart dans l'inactivation de la thromboplastine tissulaire par l'inhibiteur sérique a été examiné dans un système adéquat. Après avoir reconfirmé l'indispensabilité du complexe facteur VII pour l'action de l'inhibiteur, il a été constaté que seule la proconvertine est nécessaire pour l'inactivation tandis que le facteur Stuart ne paraît pas participer à ce processus.

Biological Properties of Synthetic Bradykinin

In the course of synthetic work on polypeptides BOISSONNAS, GUTTMANN, and JAQUENOUD recently synthesized¹ a nonapeptide with the following structure: H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH.

A preliminary account of its biological activity was given in this journal² with the conclusion: 'Further work will demonstrate if it is identical with or closely similar to pure bradykinin'.

Active concentrations of different bradykinins on various biological structures.

	Synthetic bradykinin ⁵	Natural bradykinin by the action of	
		Trypsin ⁶	<i>Bothrops</i> venom ⁷
Isolated guinea pig ileum	1 ng/ml	1 ng/ml	1 ng/ml
Isolated rat uterus	0.03 ng/ml	0.1 ng/ml	0.1 ng/ml
Blood pressure in the anaesthetized cat	500 ng/kg	400 ng/kg	30 ng/kg
Capillary permeability by intradermal injection in the guinea pig	1 ng	1 ng	10 ng

Since then ELLIOTT, LEWIS, and HORTON have, in view of the biological properties of this synthetic nonapeptide, reinvestigated their proposed structure for natural bradykinin³ and, after new degradation studies, provided evidence⁴ that natural bradykinin is a nonapeptide having the structure depicted above. Therefore, the previously reported synthetic nonapeptide^{1, 2} is in fact synthetic bradykinin.

Further experimental work with this synthetic bradykinin by KONZETT and STÜRMER⁵ has revealed that it behaves like pure natural bradykinin⁶ in several tests. From a comparison of the quantitative data, some of which are given in the Table, it can be seen that the values for the activity of synthetic bradykinin and of natural pure bradykinin obtained from ox plasma by the action of trypsin are identical within the limits of biological variations. The conclusion therefore follows that, in its biological activity, synthetic bradykinin is identical with natural bradykinin obtained from ox plasma by the action of trypsin.

Data on the biological characteristics of pure natural bradykinin obtained from ox plasma by the action of *Bothrops jararaca* venom, which were reported by JAKES and MEIER⁷, have been included in the Table. They correspond reasonably well with the figures for the other natural bradykinin and for synthetic bradykinin. With regard to biological activity all three bradykinins behave similarly and cannot be distinguished from one another.

The synthesis of bradykinin opens up the possibility of using a pure and chemically defined polypeptide of high biological activity in medical research.

H. KONZETT⁸ and R. A. BOISSONNAS

Pharmakologisches und pharmazeutisch-chemisches Laboratorium der Sandoz AG., Basel, August 22, 1960.

¹ R. A. BOISSONNAS, ST. GUTTMANN, and J.-P. JAQUENOUD, *Helv. chim. Acta* **43**, 1349 (1960).

² R. A. BOISSONNAS, ST. GUTTMANN, J.-P. JAQUENOUD, H. KONZETT, and E. STÜRMER, *Exper.* **16**, 326 (1960).

³ D. F. ELLIOTT, G. P. LEWIS, and E. W. HORTON, *Biochem. J.* **76**, 16P (1960). These authors used throughout bradykinin obtained from ox plasma by the action of trypsin.

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⁵ H. KONZETT and E. STÜRMER, *Brit. J. Pharmacol.* (in press).

⁶ D. F. ELLIOTT, E. W. HORTON, and G. P. LEWIS, *J. Physiol.* **150**, 6P (1960).

⁷ R. JAKES and R. MEIER, *Exper.* **16**, 371 (1960).

⁸ Present address: Pharmakologisches Institut der Universität Innsbruck (Österreich).

Zusammenfassung

Das synthetische Bradykinin H-L-Arg-L-Pro-L-Pro-Gly-L-Phe-L-Ser-L-Pro-L-Phe-L-Arg-OH hat (innerhalb der durch Unterschiede in der Methodik bedingten Fehlerbreite) die gleiche biologische Wirksamkeit wie natürliches, durch Einwirkung von Trypsin oder *Bothrops jararaca*-Gift aus Rinderplasma gewonnenes Bradykinin.

The Effect of Uncoupling Agents on Metabolism of Insect Muscle

It was stated by SACKTOR and CHANCE¹ that uncoupling agents are without effect on oxygen consumption of isolated mitochondria of insect muscle. We found, however, that 10⁻³ M 2,4-dinitrophenol has a pronounced effect on metabolism of the intact muscle of the American cockroach². In denervated fibres, the effect was much smaller, which could not be accounted for by any changes in the activities of enzymes of different types. We will now present further data about the action of 2,4-dinitrophenol and another uncoupling agent dicumarol on muscles of *Periplaneta americana* L. and *Locusta migratoria* L.

The experiments were carried out on the intact muscle preparations described by GILMOUR³ and KUBIŠTA⁴. The oxygen consumption was measured in Warburg respirometer and glycogen determined by anthron method. Both agents were prepared in physiological solution. The preparations were perfused with some drops of these solutions which were injected into the distal parts by means of a syringe. The oxygen consumption was measured for half an hour immediately on injection. In the case with high concentrations of 2,4-dinitrophenol, the oxygen consumption was highest at the beginning of the experiment and began to decrease after 10 min.

In anaerobic experiments, the uncoupling agents were injected 5 min after the beginning of the anaerobiosis; in this period, all oxygen is supposed to be removed.

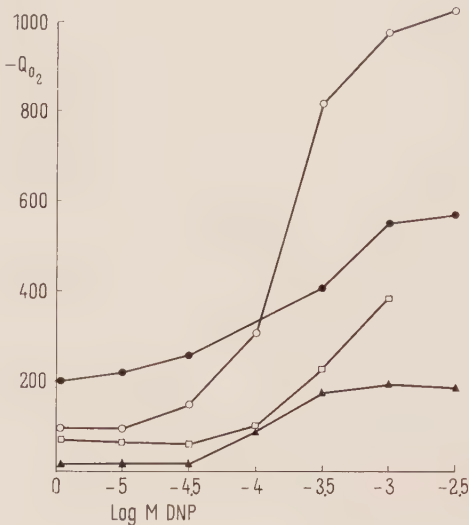
As may be seen from the Figure 2,4-dinitrophenol has a pronounced effect on the oxygen consumption of intact insect muscle preparations. The oxygen consumption is enhanced in both types of muscle fibres, i. e. in red muscles of the cockroach and also in white muscles of the locust which appear to have smaller resting values than cockroach muscles. The concentrations indicated in the Figure concern the injected solutions; the effective concentration within the muscle fibres was approximately calculated from the amount of 2,4-dinitrophenol determined analytically in the preparation and was found to be 50–60% of the concentration of the injected solution, provided that the dinitrophenol is equally distributed in the tissue.

24 days after nerve section, however, the effect of 2,4-dinitrophenol in the cockroach muscles is decreased. The differences of resting values between normal and denervated muscles are probably caused by the spontaneous activity observed in these muscles by BERÁNEK and NOVOTNÝ⁵.

Dicumarol, another uncoupling agent, also caused an increase in oxygen consumption, but its effect was smaller (Fig.) and no differences were observed between normal and denervated muscles as in the case of 2,4-dinitrophenol.

This effect of the uncoupling agents does not seem to be due solely to their action in the mechanism of oxidative phosphorylation, since both agents brought about an enhancement of metabolism in anaerobic condition (Table). The glycogen breakdown was increased particularly with 2,4-dinitrophenol. This corresponds to the findings of RONZONI and EHRENFEST⁶ in frog muscle.

It appears, therefore, that the conditions for the action of uncoupling agents are more favorable in intact muscle fibres than in isolated mitochondria. Chronic denervation causes some unknown change in biochemical equipment of muscle which results in decreased sensitiveness to 2,4-dinitrophenol.



The effect of uncoupling agents on oxygen consumption. 2,4-Dinitrophenol: ○ = normal cockroach muscles; ● = denervated cockroach muscles; ▲ = locust muscles; dicumarol: ◻ = cockroach muscles. Oxygen consumption expressed in 100 mm³/g/h

The effect of uncoupling agents on anaerobic breakdown of glycogen in muscles of the cockroach. Temperature 22°C. Duration of anaerobiosis 20 min. Glycogen expressed in mg%

Uncoupling agent	Decrease of glycogen
None	172 ± 55
10 ⁻⁴ M 2,4-Dinitrophenol	276 ± 45
10 ⁻³ M 2,4-Dinitrophenol	346 ± 85
10 ⁻³ M Dicumarol	246 ± 39

I. NOVOTNÝ and V. KUBIŠTA

Zoological Institute of Charles University, Prague (Czechoslovakia), February 15, 1960.

Zusammenfassung

Der Einfluss von 2,4-Dinitrophenol und Dicumarol auf intakte Präparate von Insektenmuskeln wurde geprüft. Beide Stoffe, besonders aber 2,4-Dinitrophenol, riefen eine erhebliche Steigerung des aeroben und anaeroben Stoffwechsels hervor. Dauernde Denervation der Muskeln führt zu erniedrigter Empfindlichkeit gegen 2,4-Dinitrophenol.

¹ B. CHANCE and B. SACKTOR, Arch. Biochem. Biophys. 76, 509 (1958).
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⁴ V. KUBIŠTA, Acta Soc. zool. bohemoslov. 20, 188 (1956).
⁵ R. BERÁNEK and I. NOVOTNÝ, Physiol. bohemoslov. 8, 87 (1959).
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Cholinesterase Activity in Skeletal Muscle After Botulinum Toxin

Following section of the cholinergic nerves to various organs, including skeletal muscle, a decreased cholinesterase activity has been found (STRÖMBLAD¹). It was therefore of interest to study whether blocking of the transmission in cholinergic nerves by botulinum toxin would also affect the cholinesterase activity.

The toxin has no acute effect on cholinesterase activity (BURGEN *et al.*²).

Methods. The effect of Botulinum toxin A on the weight and cholinesterase activity of the anterior tibial muscles of 12 months old female rats was studied. The toxin was infiltrated intramuscularly in four series of rats, each consisting of five animals. The doses used in the four series were: 0.005, 0.01, 0.02, and 0.04 μ g of toxin into each leg. In addition, in one series of five rats, the sciatic nerve in the thigh was cut aseptically under ether and in another series of five rats both botulinum toxin 0.02 μ g was given and the nerve cut. The control series consisted of 11 normal rats.

Fourteen days after the toxin had been given, or the nerve cut, the animal was killed in ether. The right and left anterior tibial muscles were removed, cleaned, washed

giving final reaction concentrations of 0.06 and 0.2% respectively. The gas phase was 5% CO₂ in nitrogen. Readings were taken, after tipping of the substrates, every 5 min for 30 min. Corrections were made for changes in a thermobarometer and enzyme blank as well as for non-enzymic hydrolysis of the substrates. All estimations were made in duplicate.

Results. When the figures for rats given toxin (Table I) are compared with the corresponding ones in the series of normal rats, it is found that the injection caused a reduction in weight and in cholinesterase activity expressed on a whole muscle basis. If the figures for cholinesterase activity are expressed on a weight basis, there is no decrease. Obviously the atrophy has been of the same order as the decrease in cholinesterase activity in the muscle. Comparisons between the four series given toxin show that the smallest dose had less effect than the other ones. The three bigger doses have in general caused the same effects; there is no indication of a greater effect when the dose was increased. The highest dose given seemed to be also the highest tolerable. The rats given 0.04 μ g in each leg were, at the end of the experimental period, in a very bad general condition.

After section of the sciatic nerve, there was an atrophy of the muscles and a decreased cholinesterase activity.

Tab. I. Weight and cholinesterase activity in anterior tibial muscles

The figures are given with $\pm$ S. E. Each value in the experimental series was compared with the corresponding one in the series of normals. The significance of a difference is given by ^a = $P < 0.05$, ^b = $P < 0.01$, and ^c = $P < 0.001$. Ach = acetylcholine as substrate. Mch = methacholine as substrate.

Procedure	Weight in mg	μ l CO ₂ /30 min/2 muscles		μ l CO ₂ /30 min/g	
		Ach	Mch	Ach	Mch
Normal muscle ($n = 11$ for figures under weight and Ach, $n = 8$ for figures under Mch)	850 $\pm$ 45	339 $\pm$ 24	186 $\pm$ 24	407 $\pm$ 34	222 $\pm$ 38
0.005 μ g of botulinum toxin A in each leg 14 days previously ($n = 5$)	660 $\pm$ 39 ^b	268 $\pm$ 40	132 $\pm$ 23	395 $\pm$ 36	196 $\pm$ 23
0.1 μ g of botulinum toxin A in each leg 14 days previously ($n = 5$)	370 $\pm$ 21 ^c	149 $\pm$ 23 ^c	74 $\pm$ 9 ^b	399 $\pm$ 51	202 $\pm$ 28
0.02 μ g of botulinum toxin A in each leg 14 days previously ($n = 5$)	333 $\pm$ 29 ^c	211 $\pm$ 35 ^b	92 $\pm$ 16 ^b	632 $\pm$ 79 ^a	272 $\pm$ 33
0.04 μ g of botulinum toxin A in each leg 14 days previously ($n = 5$)	480 $\pm$ 20 ^c	188 $\pm$ 23 ^c	87 $\pm$ 10 ^b	399 $\pm$ 62	182 $\pm$ 27
Section of sciatic nerve 14 days previously ($n = 5$)	514 $\pm$ 13 ^c	103 $\pm$ 11 ^c	48 $\pm$ 7 ^c	200 $\pm$ 22 ^c	92 $\pm$ 13 ^b

Tab. II. Weight and cholinesterase activity in anterior tibial muscle after injection of botulinum toxin, section of the sciatic nerve, or after a combination of these two procedures.

The figures after nerve section and after toxin plus nerve section are compared with those after toxin only. The significance of a difference is given by ^a = $P < 0.05$, ^b = $P < 0.01$, and ^c = $P < 0.001$.

Procedure	Weight in mg	μ l CO ₂ /30 min/2 muscles		μ l CO ₂ /30 min/g	
		Ach	Mch	Ach	Mch
0.02 μ g of botulinum toxin A in each leg 14 days previously ($n = 5$)	333 $\pm$ 29	211 $\pm$ 35	92 $\pm$ 16	632 $\pm$ 79	272 $\pm$ 33
Section of sciatic nerve 14 days previously ($n = 5$)	514 $\pm$ 13 ^c	103 $\pm$ 11 ^a	48 $\pm$ 7 ^a	200 $\pm$ 22 ^c	92 $\pm$ 13 ^c
0.02 μ g of botulinum toxin A plus section of sciatic nerve 14 days previously ($n = 5$)	353 $\pm$ 8	87 $\pm$ 7 ^b	45 $\pm$ 10 ^a	246 $\pm$ 21 ^b	127 $\pm$ 26 ^b

in saline, weighed and homogenized together in 8 ml of Krebs' bicarbonate solution. The volume was finally adjusted to 10 ml with the buffer. 1.8 ml of the homogenate was brought into the main compartment of Warburg vessels. The substrates, in 0.2 ml in a side arm, were the chlorides of acetylcholine and metacholine in amounts

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³ R. COUTEAUX, Exp. Cell. Res., Suppl. 5, 294 (1958).

⁴ A. J. BULLER, J. C. ECCLES, and ROSAMOND M. ECCLES, J. Physiol. 150, 417 (1960).

⁵ R. STRÖMBLAD, Acta physiol. Scand. 36, 47 (1956).

The atrophy was less, but the decrease in cholinesterase more pronounced, than after a big dose of botulinum toxin (Table II). In contrast to botulinum toxin, denervation caused a decreased enzyme activity also when this was expressed on a weight basis (Table I). In the series of rats both denervated and given toxin, the atrophy was greater than after only denervation, while the enzyme activity was comparable to that after denervation (Table II).

Discussion. The results here reported show that two weeks after the injection of botulinum toxin into the anterior tibial muscle there is a decreased cholinesterase activity. The decrease is, however, less than that obtained after denervation of the muscle. The difference can not be explained by loss of enzyme held in the degenerating nerve terminals, since these are known to contain extremely small amounts of enzyme compared with the end-plate (COUTEAUX³). On denervation of the muscle in addition to toxin, the decrease was similar to that after only denervation. The toxin therefore does not seem to have any effect in itself on the enzyme, which can explain why botulinum toxin does not have the same effect as section of the nerves. Thus there seems to be some influence of the nerve on the muscle not blocked by botulinum toxin. In this connexion, it is of interest to compare the suggestion put forward that the nerve has some influence on the muscle apart from that caused by release of acetylcholine (BULLER, ECCLES and ECCLES⁴). It may also be recalled that long-continued blocking of the effect of the cholinergic nerves on salivary glands by an atropine-like agent does not cause changes in the cholinesterase activity of the gland (STRÖMBLAD⁵).

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Zusammenfassung

Bei Zufuhr von Botulinum-A-Toxin in den Muskel Tibialis anterior der Ratte war die Cholinesteraseaktivität nach zwei Wochen herabgesetzt, jedoch weniger ausgesprochen als nach zweiwöchiger Denervierung des Muskels.

Crossed Reflex Actions Evoked by High Threshold Muscle Afferents

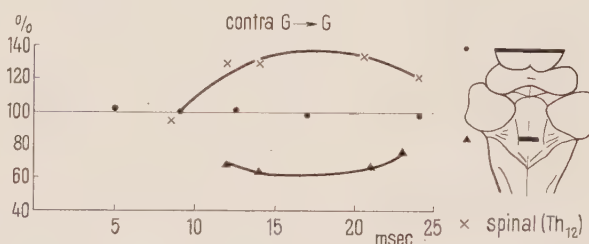
In spinal cats impulses in group II ($12-4 \mu$) and III ($4-1 \mu$) muscle afferents evoke in ipsilateral motoneurons the actions of the general flexion reflex, i.e. widespread excitation to flexors and inhibition to extensor motoneurons. It has, however, been observed that under some conditions these afferents give inhibitory effects to flexors and sometimes also excitatory to extensors¹. The present investigation is concerned with contralateral effects from these afferents. Unanaesthetized decerebrate cats with both hindlimbs denervated were used. The effect of single conditioning volleys evoked at different stimulus strengths was examined on contralateral monosynaptic test reflexes.

In acute spinal preparations, no group I effects were observed but it has been confirmed that there are usually pronounced effects by volleys in high threshold muscle afferents². Extensors as well as flexors received either inhibition or excitation but in each experiment the same effect was usually exerted on motor nuclei of synergic

muscles (extensors or flexors). These effects occurred in combinations which included all four possible variations: (1) generalized excitation or (2) inhibition or (3) excitation to flexors and inhibition to extensors or (4) finally the opposite with inhibition to flexors and excitation to extensors. The last variation was the most frequent one, and would be expected, according to Sherrington's scheme of double reciprocal innervation. In one case a reversal from inhibition to excitation occurred in flexor motor nuclei during the course of the experiment. As group II and III volleys can evoke different crossed effects in different animals under apparently identical conditions, there is, both to contralateral flexor and extensor motoneurons, one inhibitory and one excitatory pathway. Presumably the balance between these channels is labile and easily influenced, whereas on the ipsilateral side the excitatory path to flexor and the inhibitory path to extensor motor nuclei are very predominant in the spinal state.

In the decerebrate cat, the ipsilateral synaptic actions by group II and III muscle afferents may be completely suppressed³. This is due to a tonic inhibitory control from the brain stem of interneurons mediating these effects. It has now been found that a similar control is exerted on the interneurons mediating crossed group II and III effects, as is evidenced by the finding that in the decerebrate state stimulation of these fibers never evoked effects in contralateral motoneurons.

The release of supraspinal control of ipsilateral reflex arcs has been investigated after various lesions in the lower reticular formation⁴. After a medial lesion in lower pons, there is a release of the tonic control of the inhibitory path to ipsilateral extensor motoneurons and concomitantly an opening of an inhibitory path to ipsilateral flexor motoneurons. Release of the excitatory pathway to ipsilateral flexors occurs only after a more caudal lesion in the brain stem. Similar investigations have now been made on the supraspinal control of crossed actions. A low pontine lesion gave release of crossed inhibition to extensors. In the Figure a group II + III volley in the nerve to gastrocnemius-soleus had no effect on contralateral extensor motoneurons (gastrocnemius-soleus) in the decerebrate state (●). After a pontine



The effect of a conditioning volley in the nerve to gastrocnemius-soleus on the monosynaptic reflex from the contralateral gastrocnemius-soleus nerve recorded in the ventral root. 100% on the ordinate represents the unconditioned amplitude of the test reflex. Conditioned amplitude, expressed as percentage of control amplitude, is plotted as a function of time interval between the conditioning and testing stimuli. The three series of measurements were obtained after the lesions indicated in the schematic drawing, all at a conditioning stimulus strength supramaximal for group III.

¹ R. M. ECCLES and A. LUNDBERG, Arch. Biol. 97, 199 (1959).

² E. R. PERL, J. Neurophysiol. 21, 101 (1958).

³ R. M. ECCLES and A. LUNDBERG, J. Physiol. 147, 565 (1959).

⁴ B. HOLMGVIST and A. LUNDBERG, J. Physiol. 148, 70 P (1959).

lesion, there was inhibition ($\blacktriangle$), which changed into excitation after a low spinal section ($\times$). In addition the pontine lesion gave a release of inhibition to contralateral flexor motor nuclei, which sometimes remained, sometimes changed to excitation after a lesion at obex. Thus the effect of a low pontine lesion is a generalized release of inhibitory group II and III pathways to ipsilateral and contralateral extensor and flexor motoneurons with no sign of a reciprocal or a double reciprocal organization.

There are similarities in the supraspinal control of ipsi- and contralateral effects by high threshold muscle afferents which suggest a functional linkage. However, the experiments with supraspinal control have also given further evidence of the existence of two sets of pathways mediating actions of opposite modality from these afferents to contralateral extensor as well as flexor motoneurons. It is possible (as has been suggested for the ipsilateral effect⁴ that the opposite actions on a given contralateral motor nucleus are evoked by one and the same afferent system and that supraspinal centres can select either channel or close both.

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Résumé

Chez le chat spinal des impulsions provoquées dans les fibres afférentes à seuil élevé d'origine musculaire chez le chat spinal facilitent ou inhibent des motoneurons contra-latéraux fléchisseurs et extenseurs. Chez l'animal décérébré, ces actions croisées sont supprimées, tandis qu'après une lésion pontine, les actions inhibitrices se manifestent.

Observations on the Neuropathology of 'Reeler', a Neurological Mutation in Mice¹

The 'reeler' syndrome is caused by a single recessive gene mutation. The main features of the derangement caused by the 'reeler' gene are: lack of muscular coordination, balancing difficulties and tremors. Mice homozygous for the 'reeler' gene show the disturbance after the 12th day of postnatal age. They rarely survive beyond the 21st day, and we have never been able to raise any of them to breeding age. Mice heterozygous for the 'reeler' gene behave normally.

In a previous communication² we reported a 100% increase of cerebellar cholinesterase in mice homozygous for the 'reeler' gene over the values obtained for cerebellar enzyme concentration of normal littermates, who carry the 'reeler' gene either in heterozygous condition or not at all.

A study of the neuropathology of this mutation was begun with a histological analysis of brains of 'reeler' animals.

'Reeler' mice and their normally behaving littermates were sacrificed between the ages 18–21 days postnatally. Their brains were fixed in 10% formalin. The material was embedded in paraffin, cut 15 μ thick in sagittal or parasagittal plane. The sections were stained with cresyl violet.

The histological analysis revealed that the organization of the cerebellum is markedly altered in afflicted 'reeler' animals. The typical appearance of the folia is missing.



Fig. 1. Sagittal section of cerebellum of a mouse homozygous for 'reeler' gene. Age 18 days. Note: Invasion of molecular layer by various cells, reduction of granular layer and disturbance of the arrangement of Purkinje cells. $\times 80$.

The arrangement of Purkinje cells which normally surround the granular layer is severely disturbed. The granular layer is much reduced and the area of white matter contains large numbers of cells, which resemble Purkinje cells (Fig. 1 and 2).

The meaning of our findings in terms of neurophysiology is still obscure. The nature of the 'reeler' syndrome, the most dramatic symptoms of which consist of fine intentional tremors, and failure to maintain locomotor balance in homozygous animals certainly suggests cerebellar involvement.

In considering these findings it is of interest that the histological analysis of brains of the 'reeler' mutants revealed a severe disturbance of the cytoarchitectonics of the cerebellum. In a number of neurological mutants in mice, which have been investigated, no gross histological changes within the central nervous system have been detected^{3–5}. There are a few exceptions. In the 'agitans' mutation, which resembles the 'reeler' syndrome in some respects, degenerative changes limited to Purkinje cells and to a lesser degree to mossy fibers of the cerebellum of afflicted animals have been reported by MARTINEZ and SIRLIN⁶.

¹ Supported by Grant No. B-1716 from USPHS National Institute of Neurological Diseases and Blindness.

² M. HAMBURGH, *Anat. Rec.* 130, 311 (1958).

³ S. GLUECKSOHN-WAELSCH, *Progr. Neurobiol.* 4, 108 (1959).

⁴ H. GRUENEBERG, *The Genetics of the Mouse* (M. Nijhoff, The Hague 1952).

⁵ P. J. HARMAN, *Progr. Neurobiol.* 4, 96 (1959).

⁶ A. MARTINEZ and J. L. SIRLIN, *J. comp. Neurol.* 103, 131 (1955).



Fig. 2. Comparable sagittal section of cerebellum of normal littermate of the 'reeler' strain. Age 18 days. $\times 80$.

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Zusammenfassung

Eine genetisch bedingte Bewegungsstörung in der Hausmaus «reeler» wurde untersucht. Der Erbgang der «reeler»-Mutation ist einfach rezessiv. In homozygotem Zustand führt das Gen zu Gleichgewichtsstörungen und leichtem Zittern.

Das Studium der Morphologie des Gehirns der «reeler»-Mäuse ergab, dass die Cytoarchitektonik des Kleinhirns in dieser Mutante verändert und teilweise zerstört ist.

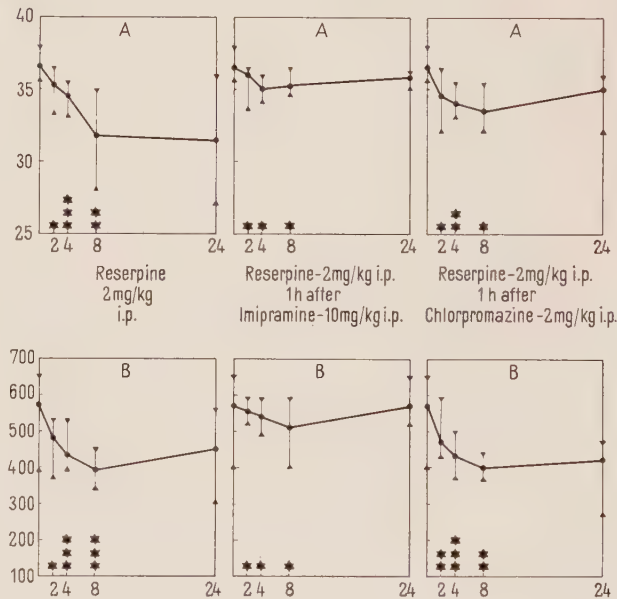
Interactions between Reserpine, Chlorpromazine, and Imipramine

The similarity between the chemical structures of imipramine [N-(γ -dimethyl-aminopropyl)iminodibenzylhydrochloride] and chlorpromazine [10-(3-dimethylamino propyl)-2-chloro phenothiazine hydrochloride] may account for some analogies in their respective pharmacological properties¹. Occasionally the therapeutic action of both drugs is comparable² but, as a rule, overactive schizophrenics are sedated by chlorpromazine while endogenous depressions are relieved by imipramine²⁻³. Experimental models for the study of the antidepressant effects of imipramine are not at present available. On the other hand differences between the action of this drug and that of other C. N. S. stimulants or antidepressants have been

described: (1) Therapeutic doses of imipramine do not attenuate the drive for food nor produces insomnia². (2) Imipramine elates depressed patients but induces neither psychomotor hyperactivity nor manic excitement in normal controls². (3) Motor activity of animals is not increased by imipramine even in doses several times greater than the therapeutic ones⁴. (4) Monoamineoxidase activity of tissue of animal given imipramine remains unaltered^{4,5}. This report deals with the effects of imipramine in rats sedated by reserpine.

Results. 10 to 15 mg/kg i. p. of imipramine given to rats before or after reserpine (up to 2.5 mg/kg i. p.) weakened certain effects of that alkaloid. Among others palpebral ptosis was less enduring and its onset was delayed. This antagonism was even greater when three doses of imipramine were given 20, 10, and 1 h before reserpine.

As presented in the Figure palpebral ptosis, hypothermia, bradycardia, and diarrhea all induced by 2 mg/kg i. p. of reserpine were antagonized by the subsequent administration of 10 mg/kg of imipramine. Other experiments revealed that the degree of this antagonism may vary in intensity. The selection of proper doses and time was critical in this respect. Chlorpromazine, 2 mg/kg i. p., given 1 h after reserpine also curtailed hypothermia and diarrhea caused by reserpine (Figure). This antagonistic effect was consistently weaker than that evinced by imipramine, and was observed only with low doses for when the dose of chlorpromazine was increased hypothermia



Interactions between reserpine, Imipramine and chlorpromazine
Abscissas = $\leftrightarrow$ Time in h after reserpine injection. Ordinates = $\leftrightarrow$ C°-[A], or pulses per minute-[B]. * = Degree of diarrhea and ptosis evaluated by an arbitrary score. Number of stars directly proportional to intensity of symptomatology. A = Rectal temperature and diarrhea. B = Heart rate and ptosis. Vertical bars = Range. Each point is the mean of at least 10 rats.

¹ R. DOMENJOZ, W. THEOBALD, Arch. int. Pharm. 120, 450 (1959).
² R. KUHN, Amer. J. Psych. 115, 459 (1958).
³ H. E. LEHMANN, C. H. COHN, and R. L. DE VERTEUIL, Canad. Psych. Ass. J. 3, 155 (1958).
⁴ E. B. SIGG, Canad. Psych. Ass. J. 4, Suppl. 75 (1959).
⁵ E. COSTA, Int. Rev. Neurobiol. 2 (1960), in press.

and diarrhea induced by reserpine were not reversed. In rats sedation caused by chlorpromazine injections was synergistic with that of reserpine. Not all the pharmacological actions of reserpine were antagonized by imipramine. In agreement with THEOBALD⁶ the blocking induced by reserpine on a previously established avoidance-escape conditioned reflex was unaffected by imipramine. Using imipramine we were unable to shorten the reserpine induced prolongation of pentobarbital hypnosis. However under different experimental conditions DOMENJOZ¹ antagonized the action of the alkaloid to prolong barbiturate narcosis. More recently SULSER⁷ *et al.* successfully used imipramine to antagonize the effects of reserpine on the depression induced by alcohol in mice. This antagonism is shown only with selected doses of alcohol. An attempt to elucidate the mechanisms involved in the antagonism of reserpine effects by imipramine was made by determining the concentrations of serotonin in brain of rats given the drugs in discussion. For comparison animals given chlorpromazine either alone or associated with reserpine were also used. The results obtained and the particular experimental conditions are presented in Table I.

Brain serotonin content of rats given imipramine 3 h prior 50 mg/kg i. p. of 5HTP (5-hydroxytryptophan) and killed after 1 h was not greater than that of animals given only 5HTP. It is therefore probable that this increase of brain serotonin was independent from an action of imipramine on synthesis and destruction of the biogenic amine. This idea receives support in the finding that serotonin concentrations of spleen, liver (Table II) lung, intestine, and kidney were unaffected by imipramine.

MARSHALL *et al.*¹⁴ found that imipramine, like reserpine¹⁵, inhibited serotonin uptake by platelets and lowered the serotonin content of platelets. However imipramine does not release tissue serotonin.

The depletion of brain serotonin caused by reserpine was not potentiated by imipramine (Table I). 15 mg/kg of chlorpromazine given 1 h prior reserpine did not prevent the depletion of brain serotonin (Table I).

To complete the study of the interactions between imipramine and serotonin the effect of imipramine on 5HTP induced diarrhea in mice¹⁶ was compared to that of chlorpromazine. *In vivo* (Table III) imipramine was a weaker antagonist of serotonin than chlorpromazine. The latter

Tab. I

Drug injected ^a mg/kg i.p.	Brain Serotonin ^b ng/g	
	Bioassay ^c	Spectrofluorimetric Assay ^d
Saline	241 (285–197) (25)	470 (486–456) (50)
Reserpine 1.0	160 (191–120) (12)	270 (312–288) (8) ^e
Reserpine 2.5	142 (182–102) (12) ^e	170 (190–180) (25) ^e
Imipramine 5.0	259 (288–231) (6)	—
Imipramine 7.5	—	550 (610–490) (8)
Imipramine 10.0	516 (645–404) (16) ^e	—
Imipramine 15.0	422 (476–373) (6) ^e	700 (754–646) (8) ^e
Chlorpromazine 10.0	278 (422–147) (9)	—
Chlorpromazine 15.0	486 (686–286) (6)	530 (574–486) (16)
Imipramine 15.0 + Reserpine 1.0	270 (303–237) (11)	300 (333–267) (8) ^e
Imipramine 15.0 + Reserpine 2.5	—	320 (357–283) (8) ^e
Chlorpromazine 15.0 + Reserpine 1.0	313 (384–242) (6)	—
Chlorpromazine 15 + Reserpine 2.5	—	340 (385–295) (16) ^e

^a The time interval between injections was 1 h. ^b The animals were killed 4 h after the injections. When reserpine was injected with other drugs the animals were killed 4 h after reserpine. ^c Bioassay was performed in 200 g Wistar rats according to GARVEN⁸. ^d Spectrofluorimetric measurement of serotonin was performed in 200 g. Sprague-Dawley rats according to BOGDANSKY *et al.*⁹. ^e Significant at $P = 0.5$ level versus controls.

In parenthesis are reported the fiducial limits and the number of animals. Fiducial limits = mean + (S. E. $\times$ T) calculated at $P = 0.1$ with $n-2$ degrees of freedom¹⁰.

Imipramine and to a lesser extent chlorpromazine significantly increased brain serotonin concentrations. After single intraperitoneal injections of imipramine (15 mg/kg) the increase of brain serotonin reached its peak within 4–5 h and disappeared in approximately 8 h. Maximal effects were obtained with 10–15 mg/kg of imipramine, while greater doses were eventually less effective. The necessity to select dose and time could explain the negative results obtained by PLETSCHER¹¹. The increase of brain serotonin after imipramine was smaller than that found¹² in brain of rats given monoamineoxidase inhibitors but comparable with that found after electroshock¹³. Unlike slow acting MAOI¹¹ the rise of brain serotonin caused by imipramine was not greater in chronic (Table II) than in acute experiments.

⁶ W. THEOBALD, *Med. exper.* **1**, 102 (1959).

⁷ F. SULSER, J. WATMS, B. B. BRODIE, *Fed. Proc.* **19**, 268 (1960).

⁸ J. D. GARVEN, *Brit. J. Pharmacol.* **11**, 66 (1956).

⁹ D. F. BOGDANSKI, A. PLETSCHER, B. B. BRODIE, and S. J. UDENFRIEND, *J. Pharmacol.* **117**, 82 (1956).

¹⁰ W. J. DIXON and F. J. MASSEY, *Introduction to Statistical Analysis* (McGraw-Hill 1951), p. 157.

¹¹ A. PLETSCHER and K. F. GEY, *Helv. physiol. pharm. Acta* **17**, C 35 (1959).

¹² S. SPECTOR, P. A. SHORE, and B. B. BRODIE, *J. Pharmacol.* **128**, 15 (1960).

¹³ S. GARATTINI, *Sonderd. Schweiz. Arch. Neurol. Neurochem. Psych.* **84**, 269 (1959).

¹⁴ E. F. MARSHALL, G. S. STIRLING, A. C. TAIT, and A. TODRICK, *Brit. J. Pharmacol.* (1960), in press.

¹⁵ B. F. HUGHES and B. B. BRODIE, *J. Pharmacol.* **127**, 96 (1959).

¹⁶ D. W. WOOLLEY, *Proc. Soc. exp. Biol. Med.*, N.Y. **98**, 367 (1958).

also antagonized more efficiently than imipramine the actions of histamine⁴ and catecholamines¹.

Conclusions. The results reported in this paper demonstrate that imipramine curtails selected pharmacological actions of reserpine including brain serotonin depletion. *Imipramine* antagonizes reserpine more extensively than chlorpromazine does. Yet the latter inhibits the effects of the biogenic amines released by reserpine more promptly than imipramine^{1,4}. In cats the effects of blood-borne norepinephrine are inhibited by chlorpromazine¹⁷ but potentiated by 2 to 5 mg/kg of imipramine⁴. Greater doses of imipramine inhibited epinephrine and norepinephrine effects¹⁸. However imipramine differently from classic cocaine-like sensitizers¹⁹ fails to potentiate the response of nictitating membrane to the stimulation of cervical sympathetic postganglionic nerves¹⁷. The sedative activity of chlorpromazine and imipramine seems to be roughly correlated to their respective capacity to inhibit norepinephrine effects.

The inhibition of serotonin uptake by platelets caused by reserpine¹⁴ and imipramine¹³ suggests that these drugs impare active transport of the amine. Imipramine and reserpine also alter brain serotonin concentrations but these changes are in opposite direction. Thus a competition for identical sites of action although possible does not readily explain the interactions between reserpine and imipramine. However the possibility that the effects of imipramine on active transport of serotonin play a role in the interrelations between this drug and reserpine cannot be excluded.

In conclusion the mechanisms involved in the antagonism between reserpine and imipramine and by and large in the therapeutic action of imipramine remain practically unknown. The difference between the pharmacological effects of chlorpromazine and imipramine are at present only quantitative, but not qualitative. Chlorpromazine and imipramine are both sedative and antagonist of reserpine. In the case of chlorpromazine both activities have a comparable low threshold and the two actions almost overlap. In the case of imipramine the sedation is caused by doses greater than those antagonizing reserpine.

Tab. II

Tissue	ng/g of Serotonin	
	Controls	Imipramine ^a
Brain	(30) 309 (359–269)	(18) 445 (499–391)
Spleen	(30) 1520 (1780–1260)	(18) 1692 (2050–1334)
Liver	(30) 146 (170–122)	(18) 113 (133–93)

^a 20 mg/kg of imipramine were injected 7 times during 96 h, last injection 70 min before killing the animal. Serotonin assayed according to the biological method of GARVEN⁷.

Tab. III. Antagonism on 5-HTP Induced Diarrhea in Mice

Drug injected mg/kg i. p. 20 min before 5-HTP (mg/kg i. p.)	ED ₅₀ ^a mg/kg i. p.	ID ₅₀ mg/kg i. v.	LD ₅₀ /ED ₅₀
Chlorpromazine	3.64	50	13.73
Imipramine	20.00	38	1.94

^a According to LITCHFIELD and WILCONXON²⁰.

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Riassunto

Sia la cloropromazina che l'imipramina antagonizzano determinati effetti farmacologici della reserpina. L'effetto antagonista dell'imipramina, che come quello della cloropromazina richiede condizioni sperimentali appropriate ha un più ampio spettro. L'effetto sedativo della cloropromazina è più marcato di quello della imipramina ed è sinergico con la depressione indotta dalla reserpina.

¹⁷ S. CURVOISIER, J. FOURNEL, R. DUCROT, M. KOLSKY, and P. KOETSCHET, Arch. int. Pharmacodyn. 92, 305 (1953).
¹⁸ U. TRENDELENBURG, J. Pharmacol. 125, 55 (1959).
¹⁹ C. MORPURGO and E. COSTA, unpublished observations
²⁰ J. J. LITCHFIELD, JR., and F. WILCOXON, J. Pharmacol. 96, 99 (1949).

Hematological and Serological Investigations in Heteroparabiosis

Many investigations on experimental homoiparabiosis of adult animals have shown that parabiotic intoxication occurs between unrelated specimens¹. This intoxication in one of the partners appears in the form of strong hemolytic anemia, loss of weight, and involution of lymphoid tissues, resulting in death within two weeks². In the other parabiont, these manifestations are frequently accompanied by polycythemia and hyperplasia of lymphoid tissues. Parabiotic intoxication is due to genetic and serological differences between the partners, since the symptoms of intoxication have never been observed in parabiosis of inbred-strain mice or in mice of closely-related strains^{3–5}. CHUTE and SOMMERS⁶ confirmed the immunological basis of parabiotic intoxication by their discovery of hemagglutinins against erythrocytes of the anemic parabiont during rat parabiosis with severe hemolytic anemia in one of the partners.

This report refers to hematological and serological investigations on experimental mouse-hamster heteroparabiosis where an intensification of symptoms of parabiotic intoxication were to be expected in consequence of greater genetic and serological differences between the parabionts.

In our experiments, unrelated white mice and inbred C57 BL and CBA mice, and golden hamsters (*Mesocricetus auratus*) were used. In all, 100 heteroparabiotic pairs were carried out by uniting mice with hamsters under ether anesthesia by coelioanastomosis. Blood for investigations was taken in mice from the tail and heart, and in hamsters from the heart immediately before parabiosis and on the 4th, 7th, 10th, and 14th days during parabiosis.

¹ J. C. FINERTY, Physiol. Rev. 32, 277 (1952).
² R. E. BILLINGHAM, Science 130, 947 (1959).
³ A. SKOWRON-CENDRZAK, B. KONIECZNA-MARCZYŃSKA, and A. GROMCZAKIEWICZ, Folia Biol. 5, 117 (1957).
⁴ B. KONIECZNA-MARCZYŃSKA and A. SKOWRON-CENDRZAK, Folia biol. 7, 9 (1959).
⁵ P. KOLDOVSKY and A. SKOWRON-CENDRZAK, Folia biol., Prague 5, 322 (1959).
⁶ R. N. CHUTE and S. C. SOMMERS, Blood 7, 1005 (1952).

The number of erythrocytes and leucocytes per mm³, as well as the percentage ratio of leucocytes in smears stained by Pappenheims' method, were established. In serological investigations, the presence of hemagglutinins in the serum of parabiotic animals was detected by the agglutination test, and incomplete antibodies by Coombs' method.

In the previous experiments, KONIECZNA-MARCZYŃSKA and SKOWRON-CENDRZAK⁷ demonstrated that in 60% of cases a hemolytic anemia occurs in homoioparabiosis between unrelated white mice, causing the anemic partner to die within two weeks. In mouse-hamster heteroparabiosis, in 88% of cases severe hemolytic anemia was observed only in mice. The mean survival of mice in heteroparabiosis was 6 days. A few heteroparabiotic pairs whose mice were not affected by anemia lived for 26 days. In the majority of cases with strong hemolytic anemia in mice, hemagglutinins and incomplete antibodies were detected in the serum of hamsters. The detected antibodies had species specificity and produced agglutinations with the erythrocytes of parabiotic and non-parabiotic mice coming from different strains. In parabiotic hamsters, there were no changes in the quantities of erythrocytes in successively examined samples.

SKOWRON-CENDRZAK and KONIECZNA-MARCZYŃSKA⁸ stated leucopenia in homoioparabiosis of unrelated mice in both partners. In heteroparabiosis, a fall in the leucocyte count was observed only in hamsters on 4th day of parabiosis. This fall was statistically important and the value *t* according to the Students' test was 2.306 with the probability of error *p* = 0.05. Leucopenia during heteroparabiosis may be explained on a serological basis. Probably hamsters have no great capacity for producing leucoagglutinins against the leucocytes of the mice. Percentage changes of leucocytes were noted only in mice during heteroparabiosis. There was a quantitative increase in neutrophil granulocytes equal to that in homoioparabiosis.

In 32 heteroparabiotic pairs of the hamster-mouse type, full thickness hamster skin grafts⁹ measuring 3 × 3 cm were placed on the backs of the mice at different periods before parabiosis. It was shown that a preliminary immunisation of mice by hamster skin grafts in many cases protects them against hemolytic anemia. Only 18% of mice in heteroparabiosis developed a hemolytic anemia after preimmunisation. The mean survival period for immunised mice in heteroparabiosis was 7 days. A correlation was observed between the length of the period of preliminary immunisation before operation and the appearance of hemolytic anemia conditioning the survival time of mice in heteroparabiosis. The joining of mice in heteroparabiosis 2 or 4 days after transplantation of hamster skin led, in many cases, to hemolytic anemia or the death of the mice within 4 days of parabiosis. These data are in accordance with the results obtained in experiments on heteroparabiosis without preliminary immunisation. In heteroparabiosis carried out 7 days after skin grafting, the mice in many cases died within 3 days after operation. This fact may have been due to the coincidence of the strongest reaction in mice to hamster skin grafts and the parabiotic intoxication. In mice joined in heteroparabiosis 10 and 12 days after grafting the hamster skin, no symptoms of hemolytic anemia were observed; on the contrary, they survived longer in heteroparabiosis, from 10 to 15 days. Acquired transplantation immunity to hamster skin grafts in mice apparently induces a weaker immunological reaction to parabiotic intoxication on the part of the hamster partner.

Serum hemagglutinins in the hamster partner were observed only in one case with strong concurrent hemolytic anemia in the mouse partner.

In conclusion, it would appear that parabiotic intoxication and death of the mouse partners occur in spite of preliminary immunisation which frequently protects mice from hemolytic anemia. However, the protection thus achieved is only transient and the survival of the mouse partner is not much longer than that observed in heteroparabiosis without preliminary immunisation.

B. KONIECZNA-MARCZYŃSKA and
A. SKOWRON-CENDRZAK

Department of Experimental Zoology, Polish Academy of Sciences, Krakow (Poland), February 23, 1960.

Zusammenfassung

In der Heteroparabiose Maus-Hamster kommt eine parabiotische Intoxikation und hämolytische Anämie bei der Maus in 88% der Fälle zustande. Eine Immunisation der Maus mit Hamsterhauttransplantaten, die 10–12 Tage vor der Parabiose unternommen wird, kann die Erscheinung der Anämie bis auf 18% der Fälle herabsetzen. In Heteroparabiosen, die mit starker hämolytischer Anämie bei der Maus verliefen, wurde stets die Anwesenheit der Hämagglutinine und unvollständiger Antikörper im Serum des parabiotischen Hamsters festgestellt.

⁷ A. KELUS, B. KONIECZNA-MARCZYŃSKA, and A. SKOWRON-CENDRZAK, *Folia biol.* 5, 99 (1957).

⁸ A. SKOWRON-CENDRZAK and B. KONIECZNA-MARCZYŃSKA, *Folia biol.* 6, 175 (1958).

⁹ R. E. BILLINGHAM and P. B. MEDAWAR, *J. exp. Biol.* 28, 385 (1951).

The Influence of Thenalidine¹ on Reserpine- and Serotonin-Induced Gastric Ulcers in Rats

It was shown earlier that large doses of reserpine, administered parenterally, produce gastric hemorrhagic erosions and ulcers in rats^{2–4}. The exact mechanism of this effect is not yet clear, although some authors have suggested a certain role of serotonin and/or histamine in it^{5,6}. The administration of serotonin (5-hydroxytryptamine) in large doses produces a similar effect on the rat's gastric mucosa^{7,8}.

It was reported elsewhere that the antihistamine thenalidine (1-methyl-4-amino-N'-phenyl-N'-(2'-thenyl)-piperidine, 'Sandosten') exhibits an antiserotonin action on the isolated rat's uterus⁹, the tidal air of spinal cats¹⁰, and on the rat's paws¹¹.

¹ Trademark employed by Sandoz Pharmaceuticals is 'Sandosten'.

² E. P. BENDITT and R. L. WONG, *Amer. J. Pathol.* 32, 639 (1956).

³ J. LA BARRE and J. J. DESMAREZ, *C. R. Soc. Biol., Paris* 151, 1451 (1957).

⁴ B. I. HAVERBACK and D. F. BOGDANSKI, *Proc. Soc. exp. Biol. Med., N. Y.* 95, 392 (1957).

⁵ G. J. BLACKMAN, S. D. CAMPION, and N. F. FASTIER, *Brit. J. Pharmacol.* 14, 112 (1959).

⁶ H. W. BACHRACH, *Amer. J. digest. Dis.* 4, 117 (1959).

⁷ C. HEDINGER and F. VERAGUTH, *Schweiz. med. Wschr.* 87, 37 (1957).

⁸ G. WILHELMI, *Helv. physiol. Acta* 15, 83 (1957).

⁹ W. DOEFFNER and A. CERLETTI, *Int. Arch. Allergy, Basel* 10, 348 (1957).

¹⁰ H. KONZETT, *Brit. J. Pharmacol.* 11, 289 (1956).

¹¹ W. DOEFFNER and A. CERLETTI, *Int. Arch. Allergy, Basel* 12, 89 (1958).

The aim of this work was to find out if thenalidine can influence the gastric ulcer formation, induced by reserpine and serotonin.

Methods. Fifty albino rats, 180–220 g of body weight, were divided in 4 groups (Table). The group I was treated as a control with reserpine (Serpasil) 2 mg/kg daily i. p. throughout 3 days. The group II was also treated with reserpine in the same doses and at the same way, but 5 min before the administration of reserpine, the animals were injected with thenalidine 2 mg/kg i. p. On the fourth day, the animals of both groups were killed, their stomachs excised and examined.

30 mg/kg of serotonin(5-hydroxytryptamine creatinine sulfate) were given twice per day to the group III. The group IV was treated with the same doses of serotonin and in the same way, with a pretreatment with thenalidine 2 mg/kg s. c. 5 min before the administration of serotonin. After 24 h, the animals were killed, their stomachs excised and examined.

Results. Results are presented in the table with the following data: percentage, severity, and number of ulcers per rat, expressed in terms of Ulcer Index (U.I.)¹² in the glandular portion of stomach, and percentage of animals with hemorrhages. In the forestomach: percentage of animals with erosions.

In the first series of animals, thenalidine has produced a slight reduction of erosions and hemorrhages in the glandular portion, and an enhancement in the forestomach. However, in the second series, the animals treated with serotonin and thenalidine showed considerably less ulcers in the glandular portion (Fig. 2) than the animals treated only with serotonin (Fig. 1). The percentage of animals with hemorrhages was noticeably diminished.

Discussion. The administration of reserpine leads to a depletion of serotonin depots of the animal^{13,14}, so that reserpine-induced gastric erosions may be explained by means of such a mechanism^{4,5}. This view is supported by the findings that 5-hydroxytryptophane⁴ and serotonin^{7,8} can produce gastric ulcers. However, reserpine is known also as a histamine-releaser¹⁵. It is of interest to note that serotonin *per se* has histamine-releasing properties¹⁶. Since the application of histamine (mixed with beeswax and mineral oil) also produces gastric ulcerations¹⁷, it is possible to suppose a definite participation of this amine in the genesis of reserpine-induced ulcers.

The results presented in this paper supported the above-mentioned view. Thenalidine feebly inhibits the ulcerogenic action of reserpine, because in this case its action is related to an effect of the liberated amines on the gastric mucosa. The serotonin-induced ulcers, after pretreatment with thenalidine, have been reduced to an extent which is less than half (Table), because of the direct antiserotonin action of thenalidine. However, the occurrence of these ulcers was not completely prevented, perhaps by reason of a histamine release after the administration of serotonin, or due to an incomplete blocking effect of thenalidine.

The paradoxical effect of thenalidine on the forestomach is quite opposite to the effect of Cortisol¹². This non-glandular portion, covered with squamous epithelium, reacts otherwise than does the stomach corpus (glandular portion). It is thus obviously that the mechanisms of the ulcer production in the two parts of rat's stomach are different.

The authors wish to express their thanks to Sandoz AG., Basle, for kindly supplying thenalidine ('Sandosten').

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Department of Pharmacology, Medical Faculty, University of Skopje (Yugoslavia), February 23, 1960.

Groups	Treatment	No. of animals	Ulcer index ^a	Hemorrhages in %	Ulcers in the Fore-stomach in %
I	Reserpine 2 mg/kg i. p.	15	18,3	30	20
II	Reserpine and Thenalidine 2 mg/kg i. p.	15	14,2	26,6	40
III	Serotonin 30 mg/kg s. c.	10	18,9	87	—
IV	Serotonin and Thenalidine 2 mg/kg s. c.	10	8	60	—

^a Ulcer Index (U. I.) is calculated by adding incidence divided by 10, severity (in pluses), and number of ulcers per rat¹².



Fig. 1

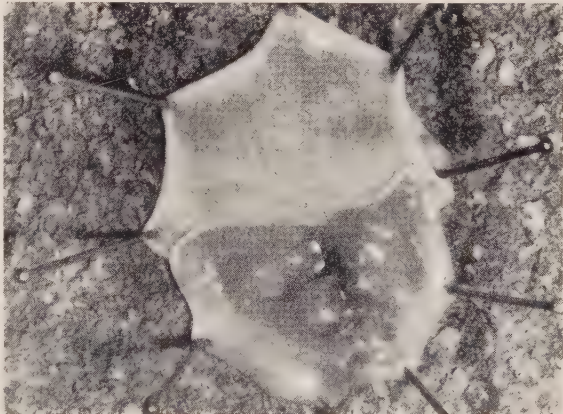


Fig. 2

¹² A. ROBERT and E. J. NEZAMIS, Proc. Soc. exp. Biol. Med., N. Y. 98, 9 (1958).
¹³ A. PLETSCHER, A. P. SHORE, and B. B. BRODIE, Science 122, 374 (1955).
¹⁴ B. B. BRODIE, A. PLETSCHER, and A. P. SHORE, Science 122, 968 (1955).
¹⁵ T. P. WAALKES and H. WEISSBACH, Proc. Soc. exp. Biol. Med. N. Y. 93, 394 (1956).
¹⁶ W. FELDBERG and N. A. SMITH, Brit. J. Pharmacol. 8, 406 (1953).
¹⁷ J. L. HAY, L. R. VARCO, F. C. CODE, and H. O. WANGENSTEEN, Surg. Gynec. Obstet. 75, 170 (1942).

Zusammenfassung

Die Vorbehandlung mit Thenalidin (Sandosten) führt zur Verminderung der mit Serotonin hervorgerufenen hämorrhagischen Erosionen im Magen der Ratten. Thenalidin zeigt einen Effekt auf das mit Reserpin bewirkte Magengeschwür und verstärkt die Vormagengeschwürbildung bei den Ratten.

Effect of the Non-Protein Fraction of Rabbit Hypothalamic Extract on the Function of Adenohypophysial Autografts in the Anterior Chamber of the Eye of Hypophysectomized Rats

It was found in previous experiments¹ that the non-protein fraction of aqueous extract of rabbit hypothalami increased acid phosphatase activity in rat pituitary homogenates. Evidence was also submitted indicating the existence of a relationship between pituitary acid phosphatase activity and the formation of thyrotrophic hormone (TSH), and suggesting that the hypothetical hypothalamic factor activating pituitary acid phosphatases may be a hypothalamic TSH-hypophysi-trophic factor².

Experiments were carried out *in vivo* to verify this assumption. Since the systemic (intravenous) administration of even relatively high doses of extract does not significantly influence thyroid function in intact rats³, evidently because this method does not give a sufficiently high concentration of the factor in the hypophysial portal vessel blood, the problem was approached by the local administration of the non-protein fraction of hypothalamic extract to adenohypophysial autografts in the anterior chamber of the eye of hypophysectomized rats.

Male rats (descendants of the Wistar strain, Velaz, Prague) acclimatized at 25 ± 2°C and fed on a standard Larsen diet with water *ad libitum*, were hypophysectomized by the parapharyngeal route. About one third of the pituitary (adenohypophysis) was aspirated into a No. 24 intramuscular needle with blunted point. An incision of about 2 mm was made with a fine scalpel in the cornea of the upper half of the left eye. The aspirated adenohypophysial tissue was then introduced into the

with autografts the eye was subjected to histological examination. The results of this examination will be given in a separate report.

There were four groups of animals: I—15 controls, II—12 hypophysectomized animals, III—10 hypophysectomized animals with autografts, to which (in the anterior chamber of the eye) 0.02 ml physiological saline was administered daily from the first to the seventh day after transplantation, IV—15 animals with autografts, to which (in the anterior chamber of the eye) 0.02 ml aqueous solution of the non-protein fraction of rabbit hypothalamus, prepared by the method described in a previous communication¹, was administered daily. 1 ml solution contained the equivalent of two rabbit hypothalami. One day after the seventh injection, all the animals were killed by exsanguination and the thyroids, adrenals, testes, and seminal vesicles were weighed.

The results are shown in the Table. Hypophysectomy was followed by plainly evident involution of all the organs in question, while autotransplantation of the adenohypophysis partially inhibited this involution. Administration of hypothalamic fraction to the autografts discernibly limited the involution of the thyroid (thyroids of the group IV were significantly— $p < 0.01$ —heavier than those of group III). Involution of the other organs (adrenals, testes, seminal vesicles) was the same or greater in group IV than in group III, however. The 'weight of the adrenals: weight of the thyroid' quotient was 2.34 ± 0.16 in group III and 1.65 ± 0.14 in group IV; $p < 0.01$.

It appears that the local application of the hypothalamic fraction stimulated TSH-secretion by the adenohypophysial autografts and did not change, or possibly inhibited, secretion of ACTH and gonadotrophins. This is in agreement with findings mentioned on relationships between the hypothalamic factor, activating pituitary acid phosphatases *in vitro* (which is present in the non-protein fraction of the hypothalamic extract) and secretion of TSH by the adenohypophysis. The possibility that its action is non-specific cannot yet be excluded.

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Group	I Controls	II Hypophysectomized	III Hypophysectomized + autograft	IV Hypophysectomized + autograft + hypothalamic fraction
No. of animals	15	12	10	15
Initial weight g	235	248	232	244
Final weight g ± σ	247 ± 5	207 ± 8	204 ± 5	205 ± 7
Thyroids mg% ± σ _M	7.00 ± 0.37	4.50 ± 0.37	4.92 ± 0.38	6.42 ± 0.39 ^a
Adrenals mg% ± σ _M	14.90 ± 0.64	9.50 ± 0.36	11.1 ± 0.68	9.60 ± 0.42 ^b
Testes g% ± σ _M	1.05 ± 0.04	0.88 ± 0.03	1.04 ± 0.05	0.95 ± 0.04 ^c
Seminal vesicles mg% ± σ	195 ± 15	107 ± 5	118 ± 14	96 ± 7 ^d
Quotient 'weight of adrenals: of thyroids' weight	2.19 ± 0.12	2.26 ± 0.22	2.33 ± 0.16	1.56 ± 0.14 ^e

Comparison of group III and IV by Fisher's *t*-test: $p =$ ^a < 0.01; ^b 0.05; ^c 0.2; ^d 0.1; ^e < 0.01.

anterior chamber of the eye (only partial disintegration of the aspirated one-third of the adenohypophysis took place during the procedure). Only completely hypophysectomized animals were taken into account, and in animals

¹ V. SCHREIBER, M. RYBÁK, V. KMENTOVÁ, *Nature* 183, 473 (1959).
² V. SCHREIBER and V. KMENTOVÁ, *Endokrinologie*, Leipzig 38, 69 (1959).
³ V. SCHREIBER and L. KRULICH, not published.

Zusammenfassung

Tglich lokal verabreichte eiweissfreie Fraktionen des hypothalamischen Extraktes zu adeno-hypophysren Auto-transplantaten in die vordere Augenkammer hypophysektomierter Ratten hemmte die Schilddrseninvolution und stimulierte mglicherweise den Vorgang bei Nebenniere, Hoden und Samenblschen.

The Urinary Excretion of Noradrenaline and Adrenaline during Acute Alcohol Intoxication in Alcoholic Addicts

High urinary excretion of noradrenaline and adrenaline has been reported¹ in the course of delirium tremens and allied conditions. There was no correlation between the catecholamine output and the intensity of single clinical symptoms, with the presence or absence of ethyl alcohol or drugs in the blood, or with the laboratory findings other than blood pressure and pulse rate. In patients with syndrome B², however, the excretion of urinary catecholamines had a definite tendency to fall immediately after the disappearance of the blood alcohol. Therefore it was of interest to investigate, whether or not the presence of alcohol in the blood of alcohol addicts is accompanied by increased excretion of urinary noradrenaline and adrenaline independent of the above-mentioned syndromes.

Material and Method. 16 male alcoholic patients showing no acute signs of alcoholic syndromes were used as test subjects at least one week after recovery. Eight patients consumed brandy or wine, and eight other patients received intravenous infusions of ethyl alcohol ca. 5 h. The blood alcohol concentrations were determined with the method of WIDMARK⁴ in all cases. The peak level varied between 1.5⁰/₀₀ and 3.3⁰/₀₀. The urinary catecholamines were determined in 24 h collections before, during and after alcohol uptake by the method of EULER and LISHAJKO⁵.

Results. There was no statistical difference between the excretion of urinary noradrenaline and adrenaline in convalescent alcoholic addicts before, during, and after ethyl alcohol administration, and that of 12 healthy persons¹ (Table). In no case following induced alcohol intoxication could the neurological symptoms of syndrome B be detected.

Discussion. The 24-h excretion of urinary catecholamines in alcohol convalescents showed no alteration when compared with the values of healthy subjects¹. Ethyl alcohol administered during a short period neither increased the urinary excretion of noradrenaline and adrenaline nor did it induce the picture of syndrome B in these subjects, even if the doses were as high as 2,3 g/kg intravenously. The increase of urinary catecholamines occurring in acute alcohol intoxications together with SB in alcohol addicts¹, seems therefore to be connected with a longer lasting abuse of ethyl alcohol than was the case in our experiments. It is evidently not dependent on the immediate effect of alcohol on the organism but on some factors developing in the course of SB and alcohol hallucinoses. In this connection, it is of interest that alcohol given intravenously in two patients during delirium tremens and SB respectively, did not influence the patterns of the urinary excretion of catecholamines.

The excretion rate of urinary noradrenaline and adrenaline during the 5-h infusion period was found to be in the range of normal diurnal variations reported by EULER

	Healthy persons	Convalescent alcoholic addicts		
		day before experiment	day of experiment	day after experiment
Noradrenaline $\mu\text{g}/24\text{ h}$	18.0 ± 6.9	16.9 ± 7.8	17.3 ± 6.9	17.0 ± 5.7
Adrenaline $\mu\text{g}/24\text{ h}$	6.4 ± 3.1	8.2 ± 4.4	7.6 ± 2.7	8.1 ± 2.8
Urinary excretion of catecholamines in convalescent alcoholic addicts before and after acute alcohol administration.				

and LISHAJKO⁵. Since, in our experiments, the catecholamines were not measured in shorter periods, a slight transient increase such as that shown by other authors⁶⁻⁸ may have escaped notice.

E. GIACOBINI, S. IZIKOWITZ, and A. WEGMANN

Beckomberga Hospital, Stockholm-Bromma and Karolinska Institutet, Department of Physiology, Stockholm (Sweden), March 24, 1960.

Zusammenfassung

Die Katecholaminausscheidung im 24-h-Urin rekonvaleszenter Alkoholpatienten wird durch einzelne thylalkoholdosen (2,3 g/kg) nicht beeinflusst.

¹ E. GIACOBINI, S. IZIKOWITZ, and A. WEGMANN, A.M.A. J. gen. Psych., in press (1960).
² Syndrome B (SB) is the most frequent acute state following an intense or long lasting alcohol or drug abuse. It is characterized by anxiety, tremor, vasomotoric reactions, hyperhidrosis, sleep disturbances, and anorexia, but without disorientation or hallucinations (for nomenclature see IZIKOWITZ³).
³ S. IZIKOWITZ, Nord. Med. 60, 1009 (1958).
⁴ E. M. P. WIDMARK, Biochem. Z. 131, 473 (1922).
⁵ U. S. V. EULER and F. LISHAJKO, Acta physiol. scand. 45, 122 (1958).
⁶ E. S. PERMAN, Acta physiol. scand. 44, 241 (1958).
⁷ I. ABELIN, Ch. HERREN, and W. BERLI, Helv. med. Acta 25, 591 (1958).
⁸ G. I. KLINGMAN and M. GOODALL, J. Pharmacol. 121, 313 (1957).

P R O E X P E R I M E N T I S

**An Ultra-High Vacuum System
Using an Oil-Diffusion Pump
with a Non-Refrigerated Isolation Trap¹**

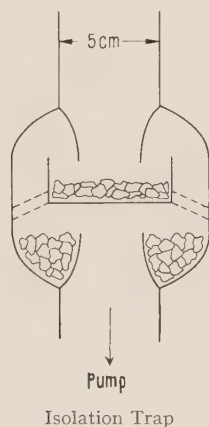
We found that an oil-diffusion pump with a modified Biondi trap² charged with activated alumina was very satisfactory in continuously maintaining ultra-high vacuum for periods of several months. The trap did not require refrigeration and the activated alumina beads could be reconditioned by bakeout. Because of the high conduct-

¹ This work was done for the Lawrence Radiation Laboratory at Livermore, California, under the auspices of the Atomic Energy Commission.
² M. A. BRONDI, Rev. Sci. instr. 30, 831 (1959).

ances of our water baffle and isolation trap the pumping speed was very high. The level of impurities in our all-glass system was negligible.

Mercury diffusion pumps have been successfully used for obtaining ultra-high vacuum (10^{-9} to 10^{-10} mm Hg) by trapping the backflowing mercury vapors in liquid nitrogen-cooled isolation traps. Such pumping systems have previously been employed in investigations on the interaction of gases with metals (BECKER³, EISINGER⁴, KISLIUK⁵, HICKMOTT⁶, and EHRLICH⁷). In our studies gases were adsorbed on the surfaces of metal ribbons, and the pressure changes in the systems were monitored with ion gauges. The amounts of adsorbed gases were determined by flashheating the metal ribbons and recording the resulting desorption pressure peaks.

The use of a mercury diffusion pump always presents the danger of mercury contamination with the consequent difficulty of reconditioning the system. In studying the interaction of hydrogen and of nitrogen with molybdenum in the dynamic system, we used a three-stage glass oil diffusion pump. After some preliminary tests with a liquid nitrogen-cooled isolation trap for preventing oil vapors from entering the system, BIONDI's non-refrigerated high-speed isolation trap was brought to our attention. As it appeared to offer great advantages, we adapted his design to our system (Fig.); we used 8 mesh activated alumina beads (Alcoa, grade F-1) as the trapping material. In addition, a high conductance water baffle was inserted between the pump and the isolation trap. Pumping speeds were obtained, on the low pressure side of the trap, of 14–24 l/sec for hydrogen in the pressure range of 1×10^{-8} to 5×10^{-6} mm Hg. This compares very well with the rated pumping speed of the oil diffusion pump which is quoted by the manufacturer as 25 l of air/s at the pump casing.



The working unit (total volume 3.3 l) as well as the isolation trap, could be baked at 450°C by swinging a hinged furnace over the system; the water baffle could be separately heated with electrical tape. In a first bakeout, for dehydrating the activated alumina beads, the temperature was slowly and stepwise increased to 450°C in two days, keeping the pressure below 1μ in the glass system, and at about 10μ in the foreline. Another two days of baking at 450°C reduced the pressure to below 0.2μ and 1μ , respectively, and the heating was discontinued. A base pressure of 5×10^{-10} mm Hg was measured at room temperature after degassing the gauges and rebaking the system at 450°C overnight. When a steady state hydrogen pressure was established in the adsorption cell, no significant amounts of impurities could be detected except after

adsorption times of several hours. A discussion of our studies has been submitted for publication elsewhere⁸.

We have operated our system continuously for a period of more than three months. Even after this interval the activated alumina bead material was still trapping oil vapors efficiently, as indicated by the low level of impurities in the system. However, a bakeout was necessitated by an inadvertent contamination of our unit with air. Ultra-high vacuum was again obtained after baking at 450°C overnight.

We would like to express our appreciation to Mr. N. MILLERON of the Lawrence Radiation Laboratory, Livermore, for this valuable advice and guidance in designing our system.

H. U. D. WIESENDANGER and R. A. PASTERNAK

Stanford Research Institute, Menlo Park (Calif.), May 2, 1960.

Zusammenfassung

Ultrahochvakuum mit Basisdruck von weniger als 5×10^{-10} mm Hg wird routinemässig erzeugt und für mehrere Monate mittels einer Öldiffusionspumpe aufrechterhalten. Eine mit aktivierter Alumina beschickte Isolationsfalle von grossem Querschnitt ist zwischen Pumpe und Glassystem eingesetzt; so wird ohne irgendwelche Kühlung das Zurückströmen von Öldämpfen in die Experimentierapparatur vollkommen verhindert.

³ J. A. BECKER, *Advances in Catalysis*, Vol. 7 (Academic Press 1955), p. 136.

⁴ J. EISINGER, *J. chem. Phys.* 29, 1154 (1958).

⁵ P. KISLIUK, *J. chem. Phys.* 30, 174 (1959).

⁶ T. W. HICKMOTT and G. EHRLICH, *J. Phys. Chem. Solids* 5, 47 (1958).

⁷ G. EHRLICH, *J. phys. Chem.* 60, 1388 (1956).

⁸ R. A. PASTERNAK and HANS U. D. WIESENDANGER, to be published.

COGITATIONES

Zur Rolle von Schwermetallionen im Wirkungsmechanismus von Strahlenschädigung und Strahlenschutz

Es ist – vor allem von BACQ *et al.*¹ – darauf hingewiesen worden, dass viele strahlenschutzwirksame Substanzen Komplexbildner sind bzw. im Organismus in solche übergeführt werden und dass auch für ausgesprochene Chelatbildner wie Komplexon oder Oxin Strahlenschutzwirkung festgestellt wurde. Dies kann als ein Hinweis dafür angesehen werden, dass an den für die Strahlenschädigung verantwortlichen Reaktionen Metallionen massgeblich beteiligt sind.

Es wird allgemein angenommen, dass diese Schädigungsreaktionen in einem Angriff von $\text{OH}\cdot$ - oder $\text{OOH}\cdot$ -Radikalen auf noch unbekannte biologische Funktionsträger bestehen und – zum Beispiel über Hydroperoxyde – zu funktionsunfähigen Folgeprodukten führen². Es ist

¹ Z. M. BACQ, A. HERVE und P. FISCHER, *Bull. Acad. Méd. Belg.* 18, 226 (1953). – P. ALEXANDER, Z. M. BACQ, S. F. COUSSENS, M. FOX, A. HERVE und J. LAZAR, *Rad. Res.* 2, 392 (1955).

² S. Z. B. Z. M. BACQ und P. ALEXANDER, *Grundlagen der Strahlenbiologie* (Stuttgart 1958), p. 73. – R. LATARJET, *Les Peroxydes Organiques en Radiobiologie* (Paris 1958).

weiterhin verschiedentlich dargelegt worden, dass zumindest ein Teil des auftretenden Strahlenschadens nicht von primär durch die Strahlung gebildeten Radikalen ausgelöst wird, sondern dass aus solchen Radikalen H_2O_2 entsteht, und dass dieses H_2O_2 das schädigende Agens darstellt³.

Für die Art der Beteiligung von Metallionen wie Fe^{3+} oder Cu^{2+} am Ablauf der Schädigungsreaktionen wäre demnach folgendes Bild zu diskutieren: Das durch die Strahlung gebildete H_2O_2 ist im Gegensatz zu den ebenfalls entstehenden Radikalen relativ langlebig. Es kann dadurch an beliebige Stellen der Zelle diffundieren und dort wieder in reaktive Radikale zerlegt werden, wenn an diesen Stellen Metallionen wie Fe^{3+} oder Cu^{2+} vorhanden sind, welche bekanntlich⁴ diesen Zerfall katalysieren.

Sind diese Metallionen nun koordinativ an Substanzen gebunden, die für das Funktionieren der Zelle wesentlich sind – wie zum Beispiel Nukleinsäuren⁵, die als «Informationsträger» für den Aufbau der Zellenzyme angesehen werden können⁶ – so ist anzunehmen, dass sie das in Form des H_2O_2 diffundierende «Radikal-Depot» räumlich gesehen gerade an solchen Stellen zu aktivieren vermögen, an denen Radikale besonders gefährliche Schädigungen hervorrufen müssen.

Zu prüfen ist demnach, ob und inwieweit Wirkungsmechanismen der genannten Art das Ausmass biologischer Strahlungsschäden bestimmen. Bei unseren Versuchen unternahmen wir zunächst, die Wirksamkeit solcher Mechanismen in einer Modellreaktion zu exemplifizieren, indem wir den Einfluss von Cu^{2+} -Ionen auf die Geschwindigkeit der Reaktionen zwischen H_2O_2 und verschiedenen Nukleotiden untersuchten.

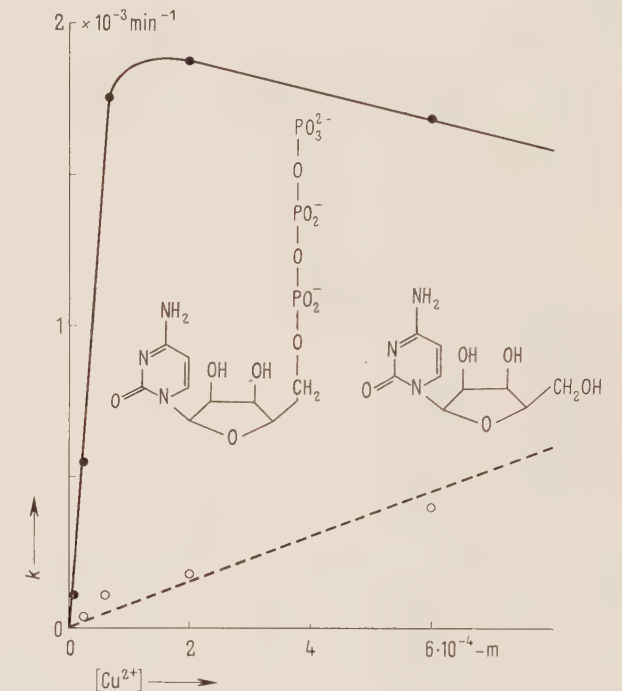
Pyrimidin-Basen wie zum Beispiel Cytidin reagieren nur sehr langsam mit H_2O_2 , die Reaktion wird jedoch durch Cu^{2+} -Ionen beschleunigt. Die Geschwindigkeit dieser Zersetzungsreaktion des Cytidins, die sich durch das Verschwinden der Pyrimidin-Absorptionsbande bei ca. 270 m μ verfolgen lässt, wurde in Abhängigkeit von der Konzentration an zugesetztem Cu^{2+} gemessen. Um den Einfluss einer Komplexbildung zwischen Substrat und Metallion zu untersuchen, wurde einmal Cytidin – welches mit Cu^{2+} unter den Versuchsbedingungen keine Komplexe bildet – und zum anderen Cytidintriphosphat (CTP) als Substrat verwendet, welches mit Cu^{2+} einen 1:1-Komplex bildet, für dessen Dissoziationskonstante ein pK von ca. 5,5 angenommen werden kann⁷. Das Ergebnis einer solchen Versuchsreihe ist in der Figur dargestellt.

Es ist ersichtlich, dass im Falle des Cytidins die Zersetzungsgeschwindigkeit nur langsam und etwa linear mit steigender Cu^{2+} -Konzentration zunimmt, während für CTP schon bei sehr kleinen, zu [CTP] etwa äquivalenten Cu^{2+} -Konzentrationen ein Maximum der Zersetzungsgeschwindigkeit erreicht wird. Diese ist hier um einen Faktor von etwa 20 grösser als die Zersetzungsgeschwindigkeit des Cytidins bei der gleichen Cu^{2+} -Konzentration.

Die Erklärung dieser Befunde ist wohl darin zu sehen, dass die im Komplexverband Substrat-Metallion aus H_2O_2 gebildeten Radikale eine hohe statistische Trefferwahrscheinlichkeit für die Reaktion mit dem Substrat haben. Für die Radikale, die im Versuch mit dem keine Komplexe bildenden Cytidin in «freier Lösung» durch Reaktion zwischen Cu-Ionen und H_2O_2 entstanden sind, werden hingegen infolge des wesentlich grösseren Diffusionsweges Konkurrenzreaktionen, wie Radikalkombinationen und Reaktionen mit H_2O_2 vor der Reaktion mit dem Substrat bevorzugt sein.

Wenn diese Vorstellungen zutreffen, sind noch krassere Unterschiede zwischen Metallionen bindenden und nicht-bindenden Substraten hinsichtlich der Reaktionsgeschwindigkeit mit H_2O_2 dann zu erwarten, wenn die Versuchs-

bedingungen biologischen Verhältnissen dadurch angenähert werden, dass der Lösung ausser dem Nukleotid noch andere organische Stoffe zugesetzt werden, die eine Radikal-abfangende Wirkung besitzen. Bei einer solchen, zu den Versuchen in der Abbildung analogen Versuchsreihe, bei welcher in der Lösung Diglycyl-Glycin (10^{-4}m) als Radikal-abfängendes «Protein-Modell» vorhanden war, ergab sich tatsächlich, dass die Zerstörungsgeschwindigkeit des CTP nur wenig beeinflusst wurde, diejenige für Cytidin jedoch durchweg um einen Faktor von $1/>10$ gegenüber den entsprechenden Versuchen ohne Peptid verringert war.



Geschwindigkeitskonstanten k für die Reaktion zwischen $10^{-3}\text{-m. H}_2\text{O}_2$ und $10^{-4}\text{-m. Cytidin (O)}$ bzw. Cytidintriphosphat (●) in Gegenwart verschiedener Cu^{2+} -Konzentrationen. k ist berechnet als Geschwindigkeitskonstante für die Reaktion pseudo-erster Ordnung in bezug auf das betreffende Nukleosid nach $d(E_{270})/dt = -k(E_{270})$. pH = 5,1 bis 5,2, $T = 37^\circ\text{C}$.

³ S. z. B. L. R. BLINKS, J. cell. comp. Physiol. 39, 11 (1952). – G. ALPER, Disc. Farad. Soc. 12, 234 (1952); Nature 172, 957 (1953). – O. WARBURG, W. SCHRÖDER, H. GEWITZ und W. VÖLKER, Naturwissenschaften 45, 192 (1958). – H. HOLZER und S. FRANK, Angew. Chem. 70, 570 (1958). – A. M. SHEFNER, Rad. Res. 12, 471 (1960).
⁴ S. z. B. H. BAXENDALE, Advanc. Catalys. 4, 31 (1952).
⁵ S. z. B. W. E. C. WACKER und B. L. VALLEE, J. biol. Chem. 234, 3257 (1959).
⁶ S. z. B. F. LEUTHARDT, Lehrbuch der physiologischen Chemie (Berlin 1959), p. 454. – A. M. KUZIN, Izvest. Akad. Nauk. S.S.S.R. Ser. Biol. 1957, 273.
⁷ Für Cu-ATP wurde $\text{pK}_1 = 5,50$ gemessen (H. BRINTZINGER und S. FALLAB, Helv. chim. Acta 43, 43 (1960)); ATP und CTP weisen nahezu identische Komplexbildungskonstanten mit den Metallionen Mg^{2+} , Mn^{2+} und Co^{2+} auf (E. WALAAS, Acta chem. Scand. 12, 528 (1958)), es ist also anzunehmen, dass eine solche Analogie auch für Cu^{2+} grössenordnungsmässig gilt.
⁸ S. z. B. J. S. WIBERG und W. F. NEUMANN, Arch. Biochem. Biophys. 72, 66 (1957). – E. FRIEDEN und J. ALLES, J. biol. Chem. 230, 797 (1958).

Die Ergebnisse der oben skizzierten Versuche zeigen also, dass in Gegenwart von Liganden durch H_2O_2 ausgelöste Zerstörungsreaktionen von Cu-Ionen dorthin gelenkt werden, wo diese koordinativ gebunden sind. In weiteren Versuchen wurde noch für eine Reihe anderer biologisch bedeutsamer Metallionen untersucht, mit welcher Geschwindigkeit CTP in Form des entsprechenden Metallion-CTP-Komplexes durch H_2O_2 zerstört wird. Die ermittelten relativen Geschwindigkeitskonstanten $k_{\text{M-CTP}}$ bezogen auf $k_{\text{Cu-CTP}} = 1$ sind in der Tabelle aufgeführt. Wie zu erwarten, sind nur Redox-wirksame Metallionen katalytisch aktiv, da die Katalyse der H_2O_2 -Zerlegung Redoxschritte des Metalls involviert⁴. Die relativen Wirksamkeiten der aktiven Metallionen Cu^{2+} , Fe^{3+} , Mn^{2+} und Co^{2+} sind im übrigen untereinander auffallend wenig verschieden.

Relative Geschwindigkeit der Zerstörung verschiedener Metallion-CTP-Komplexe durch $10^{-3} \text{ m H}_2\text{O}_2$

$k_{\text{Cu-CTP}}$	$k_{\text{Mn-CTP}}$	$k_{\text{Co-CTP}}$	$k_{\text{Fe}^{III}\text{-CTP}}$	$k_{\text{Zn-CTP}}$
1,00	0,54	0,63	1,22	0,03

Die strahlenbiologische Relevanz unserer Beobachtungen ist darin zu sehen, dass das Ausmass zumindest der auf das – durch die Strahlung gebildete – H_2O_2 zurückgehenden Strahlungsschäden zum Beispiel an Nukleinsäuren mit Sicherheit von Metallionen-Gleichgewichten abhängig sein muss. Da nun Komplexbildner in die Lage solcher Gleichgewichte im Sinne eines Metallionen-Entzugs eingreifen, ist zu verstehen, dass sie eine Prophylaxe des Strahlenschadens bewirken können.

Die Lage der für das Auftreten der Strahlungsschäden wichtigen Verteilungsgleichgewichte von Metallionen wie Cu^{2+} oder Fe^{3+} zwischen Nukleinsäuren, anderen im biologischen Medium vorhandenen Ligandsystemen und den Strahlenschutzsubstanzen ist naturgemäss in unserem Modellversuch nicht untersuchbar. Für die hochgeladenen Nukleinsäure-Anionen ist jedoch eine hohe Metallionen-Bindungstendenz zu erwarten⁸.

Wir glauben, hiermit gezeigt zu haben, dass für eine genaue Kenntnis des Zustandekommens von Strahlungsschäden noch erhebliche Informationen über die Bindungsverhältnisse katalytisch aktiver Metallionen in den biologischen Systemen notwendig sind, und dass bei *in vitro*-Untersuchungen über die Strahlenempfindlichkeit biologischer Materialien, wie zum Beispiel Nukleinsäuren, auf definierte Verhältnisse hinsichtlich des Metallionen-Gehaltes geachtet werden muss.

Herrn Professor Dr. S. FALLAB danken wir für die Diskussion verschiedener Probleme, Fräulein HEDY KULL für die Ausführung experimenteller Untersuchungen und dem Schweizerischen Nationalfonds zur Förderung der wissenschaftlichen Forschung, Kommission für Atomwissenschaften, für die gewährte Unterstützung.

H. BRINTZINGER, B. PRIJS und H. ERLMEYER

Anstalt für Anorganische Chemie, Basel, 6. Juli 1960.

Summary

The catalysis of the reaction between H_2O_2 and nucleotides by biologically important metal ions has been studied spectrophotometrically, the importance of complex-formation equilibria for this catalysis was demonstrated. Radiobiological implications are discussed.

COGITATIONES

Temperature and Salinity Relationships in Marine Bottom Invertebrates¹

By C. SCHLIEPER, H. FLÜGEL, and J. RUDOLF²

In recent times, the biological significance of temperature and salinity in sea water has been thoroughly discussed by GUNTER³ and PEARSE and GUNTER⁴. A number of other excellent reviews touch on various aspects of the effect of temperature and salinity on marine organisms⁵⁻⁸. Here we should like to restrict ourselves to a few significant observations.

In order to study the influence of temperature and salinity on the viability and the metabolisms and thus on the distribution of marine species, it is not necessary to examine intact animals. In most cases, an analysis of surviving tissue pieces is sufficient, because thermal and osmotic resistance are largely based on local qualities in the cells. In order to prove this, we have compared the cellular thermal and osmotic resistance of some bivalves from the French Mediterranean coast taken out of different depths of water.

The temperature of sea water at 0-1 m depth on the French Mediterranean coast changes in the course of the year on an average of approximately from 13° to 23°C. The species which live in this area therefore are used to a yearly temperature variation of at least 10°C. The temperature changes rapidly with increasing depth. Already at 100 m depth, there occur only slight variations between 13° and 15°C. It is therefore to be expected that the species, living in these depths, are relatively stenothermic: that means that they can only sustain slight temperature changes.

The salinity of the sea water on the French Mediterranean coast changes very little, between 37 and 38‰. In the 'Etangs', however it is different. In these lagoons, separated from the open sea, salinities from 37 to 15‰ and less do occur.

Seven species of bivalves were examined. The shore water forms, *Mytilus galloprovincialis*, *Tapes decussatus*, and *Cardium edule*, were easily obtained. *Mytilus galloprovincialis* and *Cardium edule* were obtained from the free sea shore as well as from the 'Etangs'. For purposes of comparison, *Mytilus edulis* and *Cardium edule* from the Baltic Sea were used later on. *Pinna nobilis* were obtained from medium depths by diving. *Pinna pectinata* and *Avicula hirundo* were finally dredged out of 100 m depth.

At first the cellular thermal resistance was measured on small surviving gill pieces in sea water at a temperature of 35°C and higher. The temperature was increased by 1°C every 15 min. At equal time intervals, the ciliary activity of the tissue was examined under the microscope. Typical and reproducible differences appeared. The gill cells of *Tapes decussatus* from the sand of the sea shore showed

¹ Presented at the Internat. Oceanographic Congress, New York 1959.

² Institut für Meereskunde der Universität Kiel (Germany) and Laboratoire ARAGO, Banyuls-sur-Mer (France).

³ G. GUNTER, Geol. Soc. Amer., Memoir 67, 1, 159 (1957).

⁴ A. S. PEARSE and G. GUNTER, Geol. Soc. Amer., Memoir 67, 1, 129 (1957).

⁵ O. KINNE, Ann. Biol. 33, 87 (1957).

⁶ F. E. J. FRY, Ann. Biol. 33, 205 (1957); Ann. Rev. Physiol. 20, 207 (1958).

⁷ A. REMANE and C. SCHLIEPER, Die Biologie des Brackwassers (Stuttgart 1958), p. 348.

⁸ J. VERWEY, Ann. Biol. 33, 129 (1957).

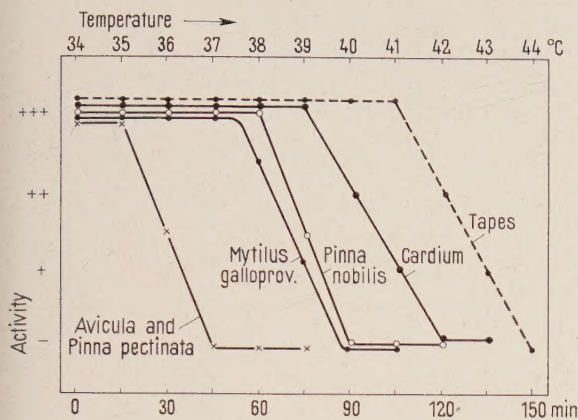


Fig. 1. Comparison of the cellular thermal resistance in the gill tissue of some bivalves from different depths.

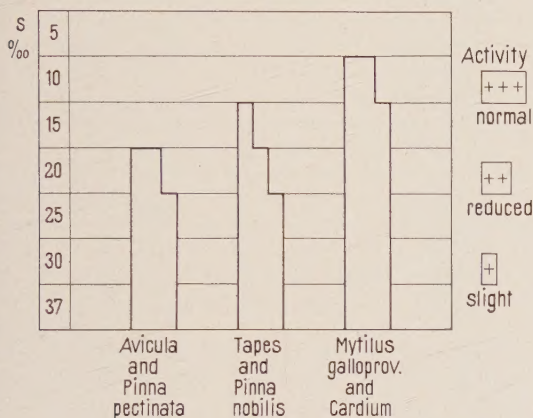


Fig. 2. Comparison of the cellular osmotic resistance in the gill tissue of some bivalves from different depths.

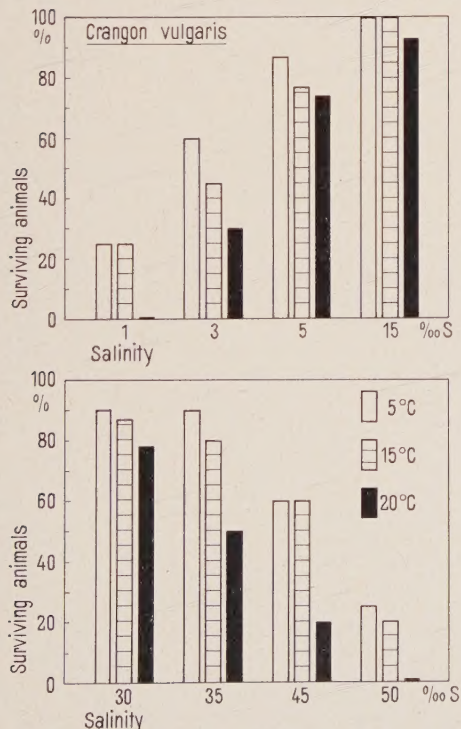


Fig. 3. Rates of survival for the shrimp *Crangon vulgaris* in sea and brackish water at different temperatures after 10 days acclimatisation.

the greatest resistance. *Cardium edule* also proved itself to be very resistant. *Mytilus galloprovincialis* and *Pinna nobilis* were a little less resistant. The bivalves *Avicula hirundo* and *Pinna pectinata* from the cool deep waters were rather sensitive to high temperatures (Fig. 1).

The same typical differences were found during other experiments in which the survival time was measured at various lethal constant temperatures.

Other experiments have shown that the salinity of the external medium influences the upper individual phenotypical temperature limit. Such observations can, of course, only be made with euryhalinic species. The tissues from *Cardium* were examined in this case. The bivalves were previously adapted to different salinities. Thereafter the individual thermal resistance had decreased with the lowering of the salinity. It is therefore necessary to work at the same salinity of sea water, if one wants to compare differences of genetic origin.

During the study of the cellular thermal resistance, the acclimation temperature must also be observed. Only when the objects have previously been acclimatised at the same temperature, can one measure comparable resistance differences. One must therefore consider the ability of individual phenotypical thermal acclimatisation. Such an ability does not exist in *Avicula hirundo* and *Pinna pectinata* living in cold deep water. But it is easy to demonstrate the individual thermal acclimatisation of the tissues in *Mytilus edulis* from the Baltic sea shore. Animals which have been acclimatised to 5°C, were, at the lethal temperature of 35°C, much less resistant than those individuals which were previously acclimatised to 20°C. If one changes the acclimatisation temperature from 5° to 20°C, or *vice versa*, then it takes approximately 4 days until the tissue has obtained the corresponding thermal resistance.

The cellular osmotic resistance can likewise be measured on small surviving gill pieces. The tissue pieces were immediately transferred to different sea water dilutions. The cell activity was controlled on the beating rate of the terminal cilia after 48 h. *Avicula hirundo* and *Pinna pectinata*, both from cold deep water, showed the least cellular osmotic resistance. The other species from higher water levels were, in comparison, much more resistant to the dilution of the external medium. But no common relationship between the thermal and osmotic resistance were found. *Tapes decussatus*, which showed by far the greatest thermal resistance, was osmotically more sensitive than *Cardium edule* and *Mytilus galloprovincialis* (Fig. 2).

Individual phenotypical differences may also appear during the examination of the osmotic resistance. The tissues of the *Mytilus galloprovincialis* from the brackish water of the 'Étangs' were plainly more resistant to the dilution of the external medium than the mussels from the more concentrated sea water of the free coast. The cellular resistance of the sea shore mussels, on the other hand, increased after being transferred into brackish water. The same observations, in the opposite sense, could be made on mussels from the 'Étangs' after being transferred into normal sea water. The individual acclimatisation of the cellular osmotic resistance to the changed salinity of the sea water, however, took up to 14 days' time. This process takes more time than the adjustment of the internal medium to the new concentration of the external medium. When the transferred mussels are already isotonic with the new external medium, after one day, the osmotic resistance bound to the colloidal structure of the protoplasm can still remain unchanged. The first signs of the resistance acclimatisation can be shown only after 2 or more days.

The process of the thermal and osmotic acclimatisation of the marine invertebrates, which possess osmoregulatory abilities, is more complicated. In this case, the osmotic resistance and the osmoregulatory performance depend largely upon the acclimatisation temperature. We have used the shrimp, *Crangon vulgaris*, in experiments with various temperatures and salinities⁹. In high and low salinities, the euryhaline shrimps are osmotically more resistant at low temperatures (Fig. 3). For a temperature range from 5° to 15°C, the optimal salinity of *Crangon* lies approximately between 15 and 30‰ salinity. At 20°C the resistance area or 'the zone of tolerance' is much smaller than at 15° and 5°C. These observations do not confirm earlier hypothetical conclusions¹⁰ that for 2-year-old shrimps the optimum salinity decreases with a fall in temperature below 21°C. If, however, one reduces the temperature of the external medium below 3°C, the osmotic resistance of *Crangon* decreases more rapidly as the temperature approaches zero. The osmoregulation of *Crangon* in brackish water apparently functions sufficiently only within a middle temperature range, between 5° and 15–20°C.

In order to analyse the influence of the temperature upon the osmoregulation of *Crangon*, we determined the freezing point depression of the blood and the external medium at 5°, 10°, and 15°C. Figure 4 shows that sea water at 27‰ salinity is nearly isotonic with long-acclimatised individuals. The difference in the freezing points of the internal and external medium is very small in this case, and is possibly not caused by any activity of the animal. At all salinities of the external medium as applied by us, the osmoregulatory performance was observed to be strongest at 5° and not at 15°C.

At temperatures below 5°C, during which, as has already been pointed out, *Crangon* in brackish water survives only with difficulty, the osmoregulatory performance also decreases! It is not easy fully to demonstrate this decrease in the osmoregulatory performance at 2.5° or 1°C. During the first days, the osmoregulatory performance decreases only slowly, and then breaks down after a certain amount of time. When this happens, the animals will die quickly. If the animals in our experiments, for instance at 1°C, after a certain amount of time, were plainly weakened in their motions and reactions, then the concentration of the blood had always greatly decreased. These observations are contradictory to the previous data by BROEKEMA¹⁰, according to which in all cases *Crangon*

in warm brackish water is supposed to show a greater osmoregulatory performance than in colder water. But BROEKEMA has measured the electrical conductivity of the blood of *Crangon*. She therefore determined only salinity changes, not the osmotic changes due to organic molecules. We used the method of freezing point depression which gives more reliable figures for the osmotic concentration. We conclude that the shrimps are unable to live in brackish estuarine waters of low salinity in winter, because their osmoregulation breaks down at temperatures below 3°C. Furthermore, we believe that our observations show that one cannot explain the heavy immigration of marine invertebrates into tropical warm brackish waters simply by the hypothesis that osmoregulation is, on the whole, easier in a warmer medium^{11,12}.

Zusammenfassung

Das Ausmass des Temperaturbereiches mariner Arten ist in erster Linie durch erbliche zellphysiologische Eigenschaften bedingt. An isolierten Gewebestücken mariner Bodenvertebraten aus kaltem Tiefenwasser und aus wärmeren oberflächlichen Schichten lässt sich dementsprechend zeigen, dass ihr genotypischer thermischer Resistenz- und Leistungsbereich quantitativ verschieden ist und bei stenothermen Arten auch nicht individuell phänotypisch verändert werden kann.

Der Umfang des Salzgehaltsbereiches stenohaliner und euryhaliner Arten ist ebenfalls in erster Linie zellulär genetisch bedingt. Jedoch beruhen thermische und osmotische Resistenz auf verschiedenen zellphysiologischen Mechanismen. Nur bei euryhalinen Formen lösen Veränderungen der Salzkonzentration des Aussenmediums individuelle zelluläre Resistenz- und Leistungsverschiebungen aus.

Niedere Temperaturen begünstigen innerhalb des art-spezifischen thermischen Resistenzbereiches Anpassung an extrem niedrige und hohe Salzkonzentrationen.

Die starke Einwanderung mariner Arten in tropische Brackwasser kann nicht durch die Annahme erklärt werden, dass die Osmoregulation in der Wärme leichter ist.

⁹ H. FLÜGEL, *Naturwissenschaften* 46, 213 (1959).

¹⁰ M. M. M. BROEKEMA, *Arch. Néerl. Zool.* 6, 1 (1942).

¹¹ C. SCHLIEFER, *Int. Oceanograph. Congress*, New York (1959), Preprints, p. 250.

¹² The authors wish to express their appreciation of the support of this work by the Universität Kiel, the Deutsche Forschungsgemeinschaft, and the Université de Paris, and in particular of the facilities and assistance so generously provided to the first author by Professor G. PETIT, Directeur, Laboratoire ARAGO, and his staff.

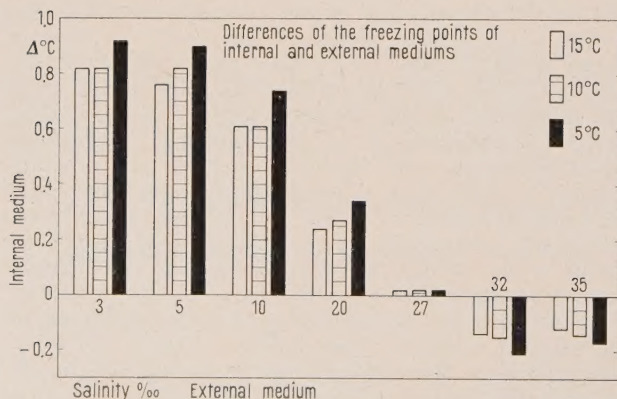


Fig. 4. The osmoregulatory performance of the shrimp *Crangon vulgaris* at various temperatures after acclimatisation in waters of different salinities.

CORRIGENDUM

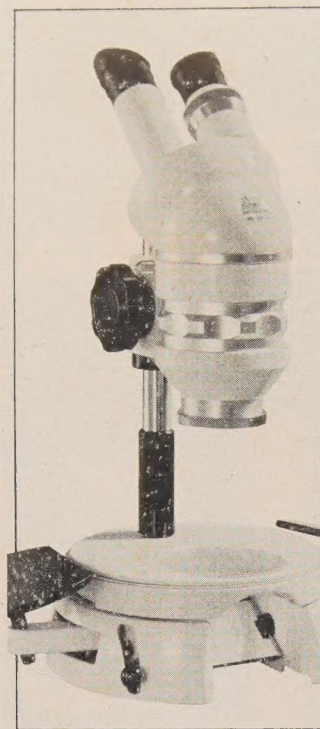
R. JAQUES and R. MEIER: *Pharmacological Characteristics of Bradykinin B*. *Exper.* vol. XVI, fasc. 8, p. 371 (1960).

The first sentence of the results (p. 371, left, 11th line from below) should read: A: 1. The isolated terminal guinea-pig ileum contracted upon addition of bradykinin B in final concentrations as low as 1 to 3×10^{-12} (g/l), i. e. 1 to 3 ng/ml.

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